

CHRONIC USE OF THREE TYPES OF
ANTIDEPRESSANTS AND THEIR EFFECTS ON
MEMORY AND ANXIETY IN MALE AND FEMALE
RATS.

A thesis submitted in partial fulfilment of the requirements for the Degree

of Master of Science in Psychology

in the University of Canterbury

by V. C. Gray

2013

Acknowledgements

Firstly I would like to thank my supervisors. Professor Rob Hughes, for providing advice and guidance throughout this process. The knowledge shared and direction given have been invaluable in getting this thesis completed. Also thanks to Dr Anthony McLean for acting as my secondary supervisor.

Thanks also go to the technicians in the Animal Lab. Neroli Harris, Silvana de Freitas Costa and Kate Freeman for the work in animal care as well as showing me the lab procedure, animal handling and drug preparation.

To my postgraduate colleagues in the Psychology department, thank you for sharing your knowledge and friendship throughout this journey. It would not nearly have been so successful without your assistance and moments of light relief throughout.

To Susan Rapley for her knowledge and advice and assisting me with the writing phase of this thesis. To May Chan for her continued support, encouragement, friendship and cooking- all of these things contributed to keeping me going when stress was at its highest. To Ashlea Dassanayake for encouraging me to keep writing and for keeping me company in the Animal Lab.

To my friends outside university, thank you for being there and supporting and sticking by me, even when I was busy and stressed. Thank you for providing fun times and breaks from university when I needed them most.

To my family, thank you for loving and supporting me throughout this journey. For encouraging me to continue and your faith in my success. To my parents, sister, aunt and grandparents for showing me your support and being there when I needed you. To my family-in-law, for supporting my partner and providing a place to unwind and great people to share those moments with.

Finally to my partner Matt. This has been an epic journey and I am so grateful to have had you with me during it. You have supported and encouraged me, both mentally and financially and have put up with me (and a lack of me!), through good times and bad. I would not have made it through without you (and Rosie) to come home to. Your love and commitment have made this thesis possible.

Table of Contents

Acknowledgements.....	ii
List of Figures	iv
List of Tables	v
Abstract	vi
Introduction.....	1
Mechanisms of action of anti-depressants	2
Drugs Investigated.....	3
Behavioural Tests Used in the Present Study.....	12
Sex Differences	18
Summary	20
Methods.....	21
Subjects	21
Housing and Feeding Procedures	21
Drugs and Rationale for Doses.....	22
Apparatus and Behavioural Measures.....	23
Results.....	25
Emergence test	25
Open Field	26
Y-Maze.....	30
Discussion	44
Summary of Results	44
Anxiety Results:	45
Memory Results	49
Sex differences:	52
Concluding remarks	54
References.....	55
Appendix 1.....	75
Appendix 2.....	76

List of Figures

Figure 1: Mean (\pm SEM) emergence latency for both sexes combined following chronic exposure to normal diet (placebo), and two doses (1= low dose, 2= high dose) each of fluoxetine, reboxetine and venlafaxine.	35
Figure 2: Mean (\pm SEM) ambulation for, A. males and females separately following chronic exposure to normal diet (placebo) and two doses (1= low, 2= high) of fluoxetine, and for B. both sexes combined following exposure to normal diet (placebo) and two doses (1= low, 2= high) each of reboxetine and venlafaxine.	36
Figure 3: Mean (\pm SEM) rearing frequency for both sexes combined following chronic exposure to normal diet (placebo), and two doses (1= low, 2= high) each of fluoxetine, reboxetine and venlafaxine.	37
Figure 4: Mean (\pm SEM) grooming frequency for, A. both sexes combined following exposure to normal diet (placebo) and two doses (1= low, 2= high) of fluoxetine, and for males and females separately following chronic exposure to normal diet (placebo) and two doses (1= low, 2= high) of B. reboxetine, and C. venlafaxine. *significantly different ($p < 0.05$) from placebo. ^a Sex difference significant ($p < 0.05$) for the placebo group.	38
Figure 5: Mean (\pm SEM) number of faecal boluses for both sexes combined following chronic exposure to normal diet (placebo), and two doses (1= low, 2= high) each of fluoxetine, reboxetine and venlafaxine.	39
Figure 6: Mean (\pm SEM) frequency of centre squares occupancy for both sexes combined following chronic exposure to normal diet (placebo), and two doses (1= low, 2= high) each of fluoxetine, reboxetine and venlafaxine.	39
Figure 7: Mean (\pm SEM) frequency of corners occupancy for, A. both sexes combined following exposure to normal diet (placebo) and two doses (1= low, 2= high) each of fluoxetine and reboxetine, and B. for males and females separately following chronic exposure to normal diet (placebo) and two doses (1= low, 2= high) of venlafaxine.	40
Figure 8: Mean (\pm SEM) percent entries of the novel Y-maze arm for, A. males and females separately following exposure to normal diet (placebo) and two doses (1= low dose, 2= high dose) each of A. fluoxetine, and B. venlafaxine, and for C. both sexes combined following chronic exposure to normal diet (placebo) and two doses of reboxetine.	41
Figure 9: Mean (\pm SEM) percent time spent in the novel Y-maze arm for, A. both sexes combined following chronic exposure to normal diet (placebo) and two doses (1= low dose, 2= high dose) of fluoxetine, and for males and females separately following exposure to normal diet (placebo) and two doses (1= low dose, 2= high dose) each of B. reboxetine, and C. venlafaxine.	42
Figure 10: Mean (\pm SEM) number of, A. entries into, and B. time spent in both Y-maze arms for both sexes combined following chronic exposure to normal diet (placebo), and two doses (1= low dose, 2= high dose) each of fluoxetine, reboxetine and venlafaxine.	43

List of Tables

Table 1: Dosage levels and rat numbers for each group for each drug.....	22
Table 2: Chi square analyses of the effects of fluoxetine, reboxetine and venlafaxine on numbers of rats that entered the novel arm first.	31

Abstract

The use of anti-depressants has become common in the past 20 years with users now taking them for longer periods than initially intended. There is concern about the cognitive effects chronic use may have. Previous research has shown cognitive deficits in depressed patients taking medications but is complicated by depressive symptoms. This study sets out to examine the effects of these drugs, without the influence of depressive symptoms, on short term memory and anxiety. 140 rats (70 male, 70 female) were given either high or low doses of three antidepressants (fluoxetine, reboxetine and venlafaxine) or placebo over a three week period, representing chronic use. The y-maze, open field test and emergence test were used to test short term memory and anxiety. Although memory deficits were found for male rats taking low doses of reboxetine and high doses of venlafaxine, a more notable result was a deficit in initial attention across all drugs and in both sexes. This finding provides evidence for the need to re-examine the cognitive effects of antidepressants in greater detail.

Chronic use of three types of antidepressants and their effects on memory and anxiety in male and female rats.

Numerous studies have shown that patients with depression often complain of memory deficits and cognitive impairments (Burt, Zembar, & Niederehe, 1995; Elliott, 1998; Veiel, 1997). However, it is not apparent whether these complaints are a symptom of depression or if they remain after symptoms have receded owing to antidepressant drug effects (Fava, 2003). It is also possible antidepressants have suppressant effects on cognitive faculties, with increases in cognition unable to be detected until after antidepressant treatment is stopped (Gorenstein, de Carvalho, Artes, Moreno, & Marcourakis, 2006).

Memory deficits have been found in patients taking Selective Serotonin Reuptake Inhibitors (SSRI) using immediate and delayed recall tasks (Wadsworth, Moss, Simpson, & Smith, 2005). This leads to speculation that the use of antidepressants can have detrimental effects, resulting in deficits in cognition.

Major Depressive Disorder (MDD) is an affective disorder characterised by periods of severe emotional despair. People with MDD are likely to feel guilt and unworthiness (Fava & Kendler, 2000). MDD is a dangerous disorder as it can lead to self-harming behaviours and, in the most extreme cases, suicide. The two most common and effective treatments currently are drug and behavioural therapies (Fava & Kendler, 2000). Anxiety is an emotional condition which is best described as the presentation of an exaggerated fear state (Rosen & Schulkin, 1998). It is characterised by hypervigilance, tension and overactivity of the autonomic nervous system (Fisher, 2007). Anxiety disorders can be extremely debilitating leading to social withdrawal and significant life disruption (Carlson, 2010). Anxiety disorders are also prone to co-morbidity with MDD and the influence of antidepressants on anxiety symptoms is important to note (American Psychiatric Association, 2000).

Mechanisms of action of anti-depressants

Early research into catecholamines found that the drug reserpine, known to deplete catecholamines, caused depression in those using it. This suggested that the depletion of catecholamines may underlie MDD (van Moffaert & Dierick, 1999). Furthermore, the first anti-depressants (Tricyclics (TCA) and Monoamine Oxidase Inhibitors (MAOI)) inhibited reuptake of the catecholamine noradrenaline (NA). It was inferred that they increased catecholamine levels in the synaptic cleft and as such increased the availability of monoamines, causing an increase in extracellular monoamine levels (Millan, Lejeune, & Gobert, 2000; van Moffaert & Dierick, 1999).

Additional research extended this theory to include the indolamine, serotonin (5HT; Coppen, 1969; van Praag & Korf, 1971). These studies provided the knowledge that MDD involves dysfunction of both the NA and 5HT neurotransmitter systems, the basis of the monoamine hypothesis as a cause of depression. It has been further suggested that rather than considering depression as an increase or decrease in neurotransmitter systems, it would be more correct to describe it as a malfunction in regulation of these systems (Brown, Steinberg, & van Praag, 1994; Delgado, et al., 1990).

Studies in primates and rodents have shown that 5HT levels increase quickly after acute SSRI administration (Anderson et al., 2005; Kreiss & Lucki, 1995; Rutter, Gundlah, & Auerbach, 1994). Hirano and colleagues (2005) found that this increase occurs within 30 minutes of oral administration, and is cumulative with long term treatment (Kreiss & Lucki, 1995). However, SSRIs need two to four weeks of administration before notable behavioural changes are observed (Wong & Licinio, 2001). This inconsistency has led to the suggestion that there are structural or functional neurological changes (which occur over a longer time period) that are necessary for behavioural changes (Wang, David, Monckton, Battaglia, & Hen, 2008).

The time delay in therapeutic effects of SSRIs is similar to the time taken for new neurons to integrate into functional circuits (van Praag et al., 2002). Both functional and neurotrophic factors are likely involved in this process (Wang et al., 2008). Neurogenesis consists of an increase in the production of neural progenitors, enhanced survival of immature granule cells, and stimulated dendritic branching (Encinas, Vaahtokari, & Enikolopov, 2006; Malberg et al., 2000; Nakagawa et al., 2002). Immature granule cells are then able to be integrated into the local hippocampal circuit and improve long term synaptic plasticity (Wang et al., 2008). These changes in neurogenesis are only shown to occur when SSRI treatment is administered chronically (Madsen et al., 2000; Malberg Malberg, Eisch, Nestler, & Duman, 2000; Santarelli et al., 2003).

Alongside the functional changes, chronic fluoxetine treatment results in an increase in growth factors, (Castrén, Voikar, & Rantamäki, 2007; Rantamäki et al., 2007; Warner-Schmidt & Duman, 2007) as well as desensitisation of the 5-HT_{1A} and 5-HT_{1B} autoreceptors (Millan, 2006). Both processes are likely to contribute to the delayed onset of SSRI action. Firstly, autoreceptors are desensitised and postsynaptic receptors are activated. Next, there is a release of growth factors causing an increase in neural progenitors and maturation of new hippocampal neurons is facilitated (Wang et al., 2008). Once this sequence has enabled a functional neurological change, the measurable changes in behaviour are observed in patients using antidepressants. Combined, these observations suggest neurogenesis as a possible neuronal mechanism for the therapeutic effects of antidepressants.

Drugs Investigated

Fluoxetine.

Fluoxetine is a potent and selective inhibitor of neuronal reuptake of 5HT (Hurst & Lamb, 2000). This selectivity for 5HT-1A receptors means it has little affinity for α 1-, α 2- and β -adrenoceptors, muscarinic, 5HT (5-HT₁, 5-HT₂), histaminergic, opioid, dopaminergic and

γ -aminobutyric acid (GABA) B receptors (Beasley, Masica, & Potvin, 1992; Benfield, Heel, & Lewis, 1986; Goodwin, 1996; Hyttel, 1994). Due to this low affinity for non-target receptors, fluoxetine has a low incidence of serious side effects resulting in lower dropout rates during maintenance therapy (Montgomery et al., 1998). The most common side effects of fluoxetine are nervous system complaints including anxiety, insomnia, drowsiness and tremor; sweating and light-headedness are also observed along with gastrointestinal complaints (such as anorexia, nausea and diarrhoea) and sexual dysfunction. These complaints are usually mild and tend to dissipate within two to three weeks (Stokes & Holtz, 1997). It has been established that 20 mg/day is effective in treating MDD (Altamura, Montgomery, & Wernicke, 1988; Stark & Hardison, 1985; Wernicke, Dunlop, Dornseif, & Zerbe, 1987). Therefore, this is the recommended starting dose with a maximum recommended dose of 60 mg/day (Hurst & Lamb, 2000).

Fluoxetine enhances 5HT neurotransmission by blocking the 5HT reuptake pump in the presynaptic neuron, which in turn increases the amount of 5HT in the synapse (Croxtall & Scott, 2010; Rivas-Vazquez, 2001). The metabolic pathway of fluoxetine first involves cytochrome p450 (CYP) 2D6 and 2C isoenzymes metabolising the drug in the liver. Fluoxetine is then transformed into its primary metabolite, norfluoxetine (Hurst & Lamb, 2000), which has similar activity to fluoxetine (Hurst & Lamb, 2000). The onset of antidepressant drug action may be caused by two contributing factors: the accumulation of plasma levels of the drug through reuptake inhibition; and changes in neural function, due to the stimulation of 5HT neuron production, produced through chronic treatment (Detke, Johnson, & Lucki, 1997).

Gibbons, Hur, Hendricks-Brown, Davis, & Mann (2012) conducted a meta-analysis of randomised double blind, placebo controlled clinical trials of fluoxetine, using data from 12 adult studies with 2,635 patients and 14,048 measurements from the Hamilton Psychiatric

Rating Scale for Depression (HAM-D). The meta-analysis determined that over a six- week period there was a 35% improvement in symptoms for fluoxetine compared with placebo. Rossi, Barraco, & Donda (2004) undertook an analysis of previous meta-analyses examining treatment efficacy of fluoxetine, using data from 9087 patients, from 87 different studies. Their results confirmed that fluoxetine is both safe and effective in treating depression, with greater efficacy than placebo from the first week of treatment. Additionally, in an eight-week randomised, double-blind, parallel-group study of patients with MDD, fluoxetine treatment (20mg) resulted in significant improvements on the HAM-D and the Montgomery-Asberg Depression Rating Scale (MADRS; Corrigan, Denahan, Wright, Ragual, & Evans, 2000). When evaluated against other anti-depressants (nortriptyline (Hashemi et al., 2012); mirtazapine (Versiani, Moreno, Ramakers-van Moorsel, & Schutte, 2005); sertraline (Van Moffaert, Bartholome, Cosyns, De Nayer, & Mertens, 1995); and fluvoxamine (Dalery & Honig, 2003)), fluoxetine was shown to have a similar level of efficacy. Comparison between fluoxetine and venlafaxine in a six-week, double-blind, randomised, placebo-controlled trial demonstrated that both drugs were significantly more effective than placebo in treating MDD (Nemeroff & Amchin, 1998). A double-blind, multicentre study of fluoxetine and sertraline treatment in MDD examined the tolerability and efficacy of these drugs after six months with significant improvements from baseline shown at the end of the trial for both treatments (HAM-D, MADRAS, Leeds Sleep Evaluation Scale, Hopkins Symptom Checklist (SCL-58), the severity and improvement scales of the Clinical Global Impression and the Batelle Quality of Life Questionnaire; Latimer et al., 1996). Significant changes were observed by the first week of treatment and by the final follow up, quality of life had significantly improved.

The effects of fluoxetine on cognition, using Digit Span (subtest from the Wechsler Adult Intelligence Scale—Revised; Wechsler, 1981) and verbal paired associates (subtest of

the Wechsler Memory Scale–IV; Wechsler, 2009) were investigated in a double blind, randomised study (Fudge, Perry, Garvey, & Kelly, 1990). Fluoxetine and trazodone were used, but no placebo group was involved. Six weeks of drug administration found no effect on memory and cognition by either drug. However, this study was heavily criticised. Stein, Jarvick, & Gorelick (1993) noted the lack of placebo group, posited that the tests used were not sensitive to antidepressant drug effects and further noted there was not an assessment of the cognitive effects of the drugs independent of depression.

Later tests of the effects of fluoxetine on cognitive and psychomotor function have likewise demonstrated no negative effects in these areas (Hindmarch, 1995; Ramaekers, Muntjewerff, & O’Hanlon, 1995; Sherwood, 1995). More recently, a study by Gorenstein and colleagues (2006) examined patients after six months of antidepressant use. They found that impairments in memory and psychomotor skills were not clinically significant and that in patients using fluoxetine, performance was at 95% of controls for most psychomotor and memory tests. They concluded that such slight differences may have little clinical relevance. Other human studies using SSRIs (Fudge et al., 1990; Hale & Pinninti, 1995; Schmitt, Kruizinga, & Riedel, 2001) have been inconclusive, although Hale and Pinninti (1995) did show cognitive improvements (testing information processing capacity using the critical flicker fusion test) in depressed patients. As such there is still much debate regarding the effects of fluoxetine on cognition.

Reboxetine.

Reboxetine is a selective Noradrenaline Reuptake Inhibitor (NRI) that is selective for NA transporters and is clinically active, well tolerated and has high efficacy as an antidepressant (Berzowski, van Moffaert, & Gagiano, 1997; Dubini, Bosc, & Polin, 1997; Melloni et al., 1984; Riva et al., 1989). Reboxetine has no significant activity at 5HT, DA, histaminergic, muscarinic, cholinergic or adrenergic receptors (Burrows, Maguire, &

Norman, 1998; Siepmann, Mück-Weymann, Joraschky, & Kirch, 2001). As such, it does not have the cardiovascular, anticholinergic and major sedative effects of other antidepressants (Holm & Spencer, 1999; Schatzberg, 2000). The clinically effective dose for reboxetine is 8-10 mg/day and 4-6 mg/day for elderly patients (Burrows et al., 1998).

Reboxetine increases availability of NA at the synapse, through inhibition, which augments the efficiency of NA neurotransmission (Montgomery & Schatzberg, 1998; Wong et al., 2000). NA systems are known to play a major role in affective disease states (Brunello & Racagni, 1998). Treatments that enhance central NA neurotransmission have potent antidepressant efficacy; conversely NA depletion can produce depression-like symptoms (Anand & Charney, 2000; Delgado & Moreno, 2000; Tanaka, Yoshida, Emoto, & Ishii, 2000). NA is synthesised as follows (Hyman & Nestler, 1993): dopa is converted to DA by dopa decarboxylase in NA neurons; DA is then converted to NA by the enzyme β -hydroxylase; in adrenergic neurons, the enzyme phenylethanolamine-N-methyltransferase further converts NA to adrenaline. Chronic administration of NRIs results in changes in receptor regulation, cell signalling and neuroplasticity (likely due to increased monoamine levels) and these changes likely contribute to the clinical effects (Caldecott-Hazard & Schneider, 1992; Millan, 2006).

Several clinical studies have confirmed that reboxetine is effective for the treatment of patients with MDD (Dubini et al. 1997; Kasper, 1999; Massana, Möllerb, Burrows, & Montenegro, 1999). The antidepressant and anxiolytic action of reboxetine is highly effective in humans (Massana 1998; Schatzberg 2000; Versiani, Mehilane, Gaszner, & Arnaud-Castiglioni, 1999). Detke and colleagues (1997) used rats and the Forced Swim Test to examine the antidepressant efficacy of low and high dose, acute versus chronic administration in both fluoxetine and reboxetine. In high doses both drugs were effective in reducing depressive behaviours for (immobility, reduction in swimming and climbing) both

timeframes, but in low doses the drugs were only effective in chronic administration. This result is supported by Cryan, Page and Lucki (2005) and mirrors the behavioural effects seen in humans, supporting the use of rats as a test paradigm. Moreover, the results suggest that the effects of the antidepressants are amplified following chronic administration, especially when using low doses.

The efficacy of reboxetine has also been studied using placebo-controlled trials. When evaluated against fluoxetine (Massana, 1998), imipramine (Berzowski et al., 1997) and desipramine (Ban et al., 1998), reboxetine had a similar level of efficacy. Furthermore, both six-week long (Burrows et al., 1998; Versiani, Amin, & Chouinard, 2000) and eight-week long (Schatzberg, 2000) trials using patients with MDD showed significantly better outcomes than placebo. Longer term studies (six-week long, followed by 46 week randomly assigned double blind placebo) into the efficacy of reboxetine showed that reboxetine had a lower relapse rate than placebo and a greater probability of maintained antidepressant response (Versiani et al., 1999). With long term use, similar efficacy is seen with imipramine and fluoxetine for the prevention of relapse (Burrows et al., 1998). Similar efficacy is also found with TCAs. Reboxetine is found to be more tolerable than imipramine, with a lower incidence of suicide and suicide attempts when compared with placebo, fluoxetine and imipramine (Ban et al., 1998; Berzowski et al., 1997; Massana, 1998; Mucci, 1997).

Early studies supported reboxetine as a treatment for the symptoms of depression however, newer research is contradictory. A meta-analysis by Eyding and colleagues (2010) examined all clinical trials of reboxetine, including unpublished trials. Using all available data provides a very different picture of reboxetine as a treatment. Of the data used, 74% was from participants of unpublished trials and overall, data from 4098 patients over 13 trials was used in the analysis. The analysis showed no significant differences between reboxetine and placebo. Reboxetine was also shown to be less effective than fluoxetine, paroxetine and

citalopram. The authors calculated that the benefit of reboxetine has been overestimated by 115% compared with placebo and by 23% compared with SSRIs.

Studies assessing the cognitive effects of reboxetine are largely positive. In healthy subjects, reboxetine is not likely to impair cognitive function, suggesting that when given to patients with MDD cognitive improvements should be seen. For example, Hindmarch (1998) conducted a study of healthy males to determine behavioural effects of reboxetine compared with amitriptyline and placebo. Psychometric measures, including tests of short-term memory, were conducted from baseline and for up to nine hours post dose. Reboxetine was found to have a trivial effect on psychomotor and cognitive function and did not interact with alcohol in regards to central nervous system function.

In a study of healthy male subjects (4mg doses of reboxetine and placebo over 14 days), reboxetine lead to autonomic dysfunction and sedation as measured by quantitative EEG and psychometric tests (Siepmann et al., 2001). However, reboxetine was found not to impair cognitive or psychomotor abilities. This provided further support for findings from Tanum (2000) that reboxetine has no significant cognitive or motor function impairment, as well as no significant cardiovascular effects or withdrawal syndrome.

Gallassi, Di Sarro, Morreale, & Amore (2006) conducted a trial of patients over 50 years old with MDD examining the effects of fluoxetine and reboxetine on cognitive deficits. Multiple tests were used, including the Wechsler memory scale (Wechsler, 1987). The MDD affected patients had poorer performance on the cognitive tests than normal controls at the start of the experiment. After six months treatment MDD patients had all improved on the cognitive tests but still underperformed compared with controls on some tasks (logical memory and paired associated learning subtests of the Wechsler memory scale (Wechsler, 1987) as well as autobiographical memory).

Venlafaxine.

Venlafaxine is a bicyclic, phenylethylamine compound which has two antidepressant properties (Horst & Preskorn, 1998). It is a 5HT and NA reuptake inhibitor and does not interact significantly with adrenergic, muscarinic, cholinergic, histaminergic, benzodiazepine or opioid receptors or inhibit monoamine oxidase (Muth et al., 1986). The action of venlafaxine is distinctive because it acts dose dependently to inhibit neurotransmitter reuptake. Venlafaxine blocks reuptake of 5HT at lower doses; 5HT and NA at medium to high doses; and 5HT, NA, and DA at the highest doses (Keltner & Folks, 2005). Clinical trials have shown that venlafaxine causes fewer anticholinergic and central nervous system adverse effects than TCAs. Common side effects include nausea, headache and sweating, similar to SSRIs (Rogóz, Dziedzicka-Wasylewska, Margas, & Maj, 1998). The effects are also dose dependant, with higher doses resulting in more reported symptoms (Redrobe, Bourin, Colombel, & Baker, 1998).

Venlafaxine XR (extended release) is currently approved for use in adults with MDD, generalized anxiety disorder (Wellington & Perry, 2001), social anxiety disorder and panic disorder (Wyeth-Ayerhurst Laboratories, 2012). The recommended dose is 75-225mg once daily (Lee & Keltner, 2006) and it has been suggested in patients with depression that treatment with venlafaxine should be continued for four to nine months after remission to prevent relapse (American Psychiatric Association, 2010). Additionally, venlafaxine is known to have significant discontinuation effects with symptoms including dizziness, headache, insomnia, nausea and nervousness (Boyd, 1998; Dallal & Chouinard, 1998; Jacobson & Weiber, 1997; Lejoyeux & Adès, 1997; Louie, Lannon, Kirsch, & Lewis, 1996; Macbeth & Rajagopalan, 1998; Parker & Blennerhassett, 1998; Raby, 1998; Ricci, Amore, & Papalini, 1998). The manufacturer recommends that for patients who have had more than one

week of treatment dosage should be tapered in order to minimise side effects; clinical trials have used reductions of 75mg at weekly intervals (Wyeth-Ayerhurst Laboratories, 2012).

Two randomised, placebo controlled, double blind studies found that venlafaxine was at least as effective as buspirone and diazepam (Balfour & Jarvis, 2000). A double blind, randomised trial showed a therapeutic response after two weeks of treatment in patients with MDD, with a significantly greater response than placebo after four weeks (Thase, 1997). A double blind comparison of venlafaxine and imipramine further indicated venlafaxine provided a greater therapeutic response at two, six and twelve months (Shrivastava et al., 1994). Meta-analysis of four placebo-controlled studies using six week treatment and twelve month follow up found at the end of follow-up the relapse rate for venlafaxine (20%) was significantly less than for placebo (34%; Entsuah, Rudolph, Hackett, & Miska, 1996).

In placebo controlled studies using healthy volunteers, venlafaxine showed a slight but significant impairment in some psychometric tests (including, the critical flicker frequency test (Smith & Misiak, 1976); divided attention test (Seidel, Cohen, Wilson, & Dement, 1985); digit symbol substitution test (Wechsler, 1981); and immediate and delayed word recall (Ghoneim, Hinrichs, & Mewaldt, 1984)). However, these results were not considered clinically significant. Studies in healthy volunteers suggest that venlafaxine at dosages of 75 to 150mg/day does not impair psychomotor performance to any clinically significant degree (Troy et al., 1995; O'Hanlon, Robbe, Vermeeren, van Leeuwen, & Danjou, 1998; Troy, Turner, Unruh, Parker, & Chiang, 1997). Administration of venlafaxine has shown small but significant impairments in vigilance in healthy patients, using the Mackworth clock test (Mackworth, 1950; O'Hanlon et al., 1998). However, dose-dependent improvements in attention, concentration, memory, fine motor activity, reaction time performance and wakefulness versus placebo have also been demonstrated in healthy volunteers (Saletu et al., 1992).

Overall, these three drugs have been confirmed as effective treatments for MDD, although more recent research has shown that this may not be the case for reboxetine. Fluoxetine is currently thought not to have detrimental effects on cognition, however this is not conclusive however as research shows both positive and negative effects on cognition. Reboxetine is not known to cause cognitive impairments in those taking it. The effect of venlafaxine on cognition is unclear. Some research shows no cognitive impairment but other data suggests small but significant deficits in cognition. This experiment aims to examine the effect of chronic exposure to antidepressants on memory by using healthy male and female rats. This will examine the direct effect of antidepressants on memory without the confounding variables associated with depression. The type of memory which will be tested in this study is short term memory, specifically that for spatial information. Short term memory is a system which allows the temporary storage and management of information needed to complete complex tasks. Spatial memory enables a person to remember locations as well as spatial relationships between objects.

Behavioural Tests Used in the Present Study

Responsiveness to brightness change (Y-Maze).

The responsiveness to brightness change paradigm (Caul & Barrett, 1973) will be administered to test spatial recognition memory (Dellu, Mayo, Cherkaoui, LeMoal, & Simon, 1992) and curiosity (Hughes & Maginnity, 2007). This two-choice brightness discrimination task is helpful for quick assessment of spatial memory (Conrad, Galea, Kuroda, & McEwan, 1996) and does not require the use of reinforcers or training (Hughes, 2004). Kivy, Earl, & Walker (1956) first demonstrated rats' responsiveness to brightness change using a T-maze. Dember (1956) also performed a similar experiment with rats having to make a choice between an unchanged and a novel arm of a Y-maze. This procedure has been used to assess

responsiveness to brightness change in rats in many experiments (e.g. Becker et al., 1992; Markowska & Łukaszewska, 1981; Poucet & Buhot, 1989).

In the present study, this paradigm involves two phases — an initial acquisition and subsequent retention trial. The brightness of one of the arms of the maze is changed between the two trials and the tendency of the rats to explore the new arm recorded by noting where the rat is in the apparatus every 3 seconds. Experiments have consistently shown that under normal conditions rats typically enter the changed arm first (Dember & Millbrook, 1956; Łukaszewska, 1978; Walk, 1960). This paradigm has been updated to include measures of repeated arm entries and time spent in the changed arm (Hughes, 2001; 2002).

Rats have a natural tendency to explore (Conrad et al., 1996) and it has been reliably shown that rats prefer exploring unfamiliar environments when allowed free moving exploration (Dember & Fowler, 1958; Hughes, 1997; Lamberty & Gower, 1992; Richman, Dember, & Kim, 1987). This exploration of the unfamiliar can also be termed ‘intrinsic exploration’ (Berlyne, 1960) or ‘novelty seeking’ (McReynolds, 1962). Intrinsic exploration is regarded as curiosity-motivated exploration of stimuli for their own sake (Berlyne, 1960) without expectation of a conventional reward (Hughes, 1997). Changing the maze arms between acquisition and retention trials means the reactions to the environment are memory dependent. For rats to show preference for the new environment, they must remember the previous characteristics of the two arms (Hughes, 1997). More recently, it has been established that the response-to-change procedure is a test of short-term spatial memory (Hughes & Maginnity, 2007).

Several studies have determined that the exploration behaviours shown are due to a motivation for exploration and not for potential gain, such as escaping from the apparatus (Horsburgh & Hughes, 1981; Hughes, 1987; Misslin & Ropartz, 1981). These experiments have involved the use of anxiogenic substances with results showing reduced exploration

when such substances are given, not increased exploration as would be expected if the animal were trying to escape the situation (Hughes, 1997). Further considerations in using this paradigm include sex differences and time for initial exposure to the maze. Male rats perform better than females on tests of spatial ability (Beatty, 1979) and female rats have been shown to habituate quicker to novelty than male rats (Hughes, 1990; Russell, 1977), which may account for some of the sex differences observed with the response-to-change procedure (Hughes, 2001). Additionally, experiments have shown exposure times of 5 minutes in a T-maze (Łukaszewska, 1978) and 2 minutes in a Y-Maze (Fowler, 1958) are needed for initial entries into the novel arm to be significant, representative of the maze details being stored in short term memory. As such, in this experiment both male and female rats will be considered and the initial exposure time for the Y-maze will be 5 minutes, significantly exceeding the exposure deemed necessary for the Y-Maze, and matching that for the T-Maze.

The behaviours measured in the responsiveness to brightness change paradigm are percentage of time spent in the novel arm, percentage of entries of the novel arm, percentage of time spent in both arms, and percentage of entries of both arms. Time spent in the novel arm indicates the level of novelty preference, and as such, memory of the previous arm brightness. Percentage of novel arm entries has been demonstrated as a measure of short-term recognition memory (Hughes, 2001), with fewer entries of the novel arm revealing impaired short-term spatial memory. Time in both arms is a measure of exploratory behaviour with greater time spent exploring the maze arms indicating minimal anxiety. Percentage entries into both arms also measures anxiety, with low levels of anxiety reflected in high levels of activity.

Open field test.

The open field test (OFT) was originally devised by Hall (Hall, 1934; Hall & Ballachey, 1932) and has been developed to measure “emotionality” in rodents (Broadhurst,

1975; Denenberg, 1969; Hall, 1934; Royce, 1977; Walsh & Cummins, 1976; Whimbey & Denenberg, 1967a; 1967b). It has become one of the most common paradigms in use (Leppänen, Ewalds-Kvist, & Selander, 2005). In the present experiment the test will also be used to assess activity (Archer, 1973).

The terms “emotionality” and “anxiety” are often used interchangeably when discussing the open field (Ramos & Mormède, 1997). Separation from a group housing environment and the open space of the apparatus are both situations likely to induce anxiety in rats (Prut & Belzung, 2003). The type of anxiety which is measured by the OFT is state anxiety, or that which is caused by an external stimulus (Belzung & Griebel, 2001; Lister, 1990), as opposed to trait or internal anxiety (Lister, 1990).

Responses displayed by rats when anxious include horizontal locomotion (ambulation), time spent in the centre of the apparatus, rearing and grooming (Prut & Belzung, 2003). The most common measures in use are defecation and ambulation (Livesey & Egger, 1970), with defecation being the most common (Broadhurst, 1976; Gray, 1987; Walsh & Cummins, 1976), most validated (Broadhurst, 1957; Whimbey & Denenberg, 1967b) and most reliable (Ivinskis, 1968; 1969). Ambulation is not as well validated (Livesey & Egger, 1970) and can be affected by age, sex, strain and previous experience (Livesey & Egger, 1970). There are two opposing views regarding the significance of this activity. One view defines high ambulation as a measure of low fear or emotionality (Hayes, 1960) and the other suggests that high ambulation is evidence of a fearful animal trying to escape (Welker, 1957; 1959). Consequently, both ambulation and defecation must be considered carefully when interpreting their significance (Whimbey & Denenberg, 1967b).

A predisposition to avoid open spaces means rats are known to prefer to remain close to the walls of the OFT apparatus (thigmotaxis; Choleris, Thomas, Kavaliers, & Prato, 2001; Leppänen et al., 2005; Prut & Belzung, 2003). Thigmotaxis has been well validated as a

measure of emotionality in rats (Treit & Fundytus, 1989; van der Staay, Kerbusch, & Raaijmakers, 1990). Thus, the starting point of the rat in the open field is important to consider, as it affects thigmotaxic behaviour (Leppänen et al., 2005). Studies have shown that thigmotaxis is increased when the subject is initially placed by a wall (Kvist & Selander, 1992) and rats also tend to stay on the side of the apparatus where they are first placed (Satinder, 1969).

The behaviours measured in the OFT are ambulation, rearing, grooming, faecal boluses and occupancy of centre and corner squares. Measuring level of ambulation is an indication of activity level which is measured by recording transitions between squares of the OFT grid. Fewer transitions between squares represent lower activity and as such, higher emotionality (Broadhurst, 1957; Campbell & Candland, 1961; Denenberg & Grotta, 1964; Escorihuela et al., 1999; Hall, 1934; Hughes & Beveridge, 1987; Liebsch, Montkowski, Holsboer, & Landgraf, 1998). Rearing is a normal rodent behaviour when exploring a new environment with lower level of rearing behaviour indicative of anxiety (Walsh & Cummins, 1976). Grooming is also a common rat response (Bolles, 1960) and an increase in this behaviour is considered a marker of increased anxiety (Moody, Merali, & Crawley, 1988). The number of faecal boluses left in the apparatus is a direct measure of anxiety with higher number of faecal boluses indicative of higher levels of emotionality (Archer, 1975; Broadhurst, 1957; Campbell & Candland, 1961; Denenberg & Grotta, 1964; Escorihuela et al., 1999; Hall, 1934; Leppänen et al., 2005; Liebsch, et al., 1998; Mechan et al., 2002). The occupancy of centre and corner squares are separate measures which examine the aversion of rats to the centre of the apparatus. High occupancy of corner squares indicates increased anxiety, while a high occupancy of centre squares represents the opposite (Hall, 1934; Prut & Belzung, 2003).

Emergence test.

The emergence test is a further measure of emotionality. It is a variation of the OFT where the rat is not handled immediately prior to the OFT, which reduces handling stress that can affect OFT measures (Paré, Tejani-Butt, & Kluczynski, 2001). The emergence test measures latency to exit from a small dark space into an unknown, open, light space. In this experiment the OFT doubles as the open, light space. Handling should not influence the emergence latency as the rat spends time in the dark box before the partition is open.

Rats prefer dimly lit areas over brightly lit ones (Crawley & Goodwin, 1980; Smythe, Murphy, Bhatnagar, Timothy, & Costall, 1996), so much so that brightly lit areas are aversive, and rats actively avoid them (Godsil & Fanselow, 2004; Slawecki, 2005). In this test, the brightly lit open field serves as both a new environment and an open space, which tests the willingness of rats to overcome an avoidance of bright, open, spaces in order to explore the new environment. Rats with a higher level of anxiety are slower to overcome their natural aversion.

The behaviour measured in this test is latency to enter the light area, measured as the time taken for the rat to emerge from the dark box into the OFT far enough that the sliding partition between the areas can be closed. This is a valid measure of anxiety (Smythe, Bhatnagar, Murphy, Timothy, & Costall, 1998; Smythe et al., 1996; Timothy, Costall, & Smythe, 1999) and is the most reliable indicator of anxiety-like behaviour when examining the effects of both anxiogenic and anxiolytic treatments (Ardayfio & Kim, 2006).

Further considerations in this paradigm include illumination levels in the OFT and sex differences. Higher levels of illumination in this paradigm result in increased anxiogenic behaviour (Costall, Jones, Kelly, Naylor, & Tomkins, 1989). Thus, the illumination in this experiment is only from overhead lights in the experiment room. Under floor illumination was trialled initially, however this was too aversive as during preliminary trials all rats failed

to emerge within 10 minutes. Archer (1975) observed that female rats are generally more active than male and Gray (1971) notes that females are also less fearful. Gray also observed that in tests of exploratory behaviour, as well as emergence tests, females show lower levels of fear and anxiety. This is supported by Archer (1973) who in a review of emotional behaviour in rodents, found that female rats and hamsters generally emerge sooner than males from a familiar environment to novel ground.

The responsiveness to brightness change paradigm, using the Y-Maze apparatus is a quick and effective method for examining changes to short term memory induced by pharmaceuticals (Conrad et al., 1996). Since conception this paradigm has been extensively tested and has become a standard and reliable test of animal spatial memory (Hughes & Maginnity, 2007). The OFT has also been well validated (Walsh & Cummins, 1976). It is easily adaptable to specific requirements of any experiment as the shape, lighting and presence of objects in the apparatus can be easily modified, and the length of a trial can also be varied (Prut & Belzung, 2003). The emergence test is a relatively simple paradigm, which is adaptable and utilisable with the OFT. It has been well validated (Smythe et al., 1998; Smythe et al., 1996; Timothy et al., 1999) providing a further measure to determine emotionality.

Sex Differences

An important factor considered in this research is sex differences. It should be implicitly understood that the results from male and female subjects would differ, especially considering human behaviour. Surprisingly, most research (especially in animals) uses only one sex— mostly males. Fortunately, consideration is now being given to exploring sex differences in many studies.

In regards to general exploratory behaviour, while there are phases of higher and lower activity with age, these periods are not sexually dimorphic (Sashkov, Sel'verova, Morenkov, & Ermakova, 2009) and the animals used in this experiment will not be in the higher and lower age phases. Males and females also do not show consistent differences in learning ability (Munn, 1950). However males are often quicker to learn spatial tasks than females (Cost, Williams-Yee, Fustok, & Dohanich, 2012). Experiments by Hughes (1999) examining novelty exploration with short exposure times, demonstrated that females have higher levels of exploratory behaviour. This was considered to be because female rats habituated to the environment faster, as sex differences dissipated when the exposure time was increased to over 30 minutes. This effect may explain why, during short-term exposure to novelty, female rats explored a T-maze more than male rats (Russell, 1977).

There are large differences in the response to stress by males and females. One stressor in the experiment is likely to be the separate housing needed for individual drug dosing. This type of housing is less severe than isolated housing, but has similarities so the effect of isolation on rats must be considered. Hatch, Wiberg, Balazs, & Grice (1963) found that four weeks of isolation was sufficient to cause both male and female rats to display more aggression, become difficult to handle and show physiological impairments (caudal dermatitis and increased weight of adrenal glands). Females are considered to be less adaptable to isolated housing than males (Harris, D'Eath, & Healy, 2008). Harris and colleagues (2008) found that isolated housing did not impair spatial ability. This demonstrates that sex differences can occur in some aspects of an experiment, but not others. Consideration will be given to which results may be due to sex differences. Interpretation of results will involve determining if and such differences are attributable to the experimental manipulation or if the difference is truly sex related.

Summary

Overall, the drugs under consideration have been deemed effective as treatments for depression, although questions have recently arisen regarding reboxetine. Current data suggest these drugs do not have a significant effect on cognition, however many animal studies have not considered chronic use, or sex related differences. Assessment of psychomotor and cognitive effects of antidepressants is important to identify possible interference in everyday activities (Siepmann et al., 2001), especially if such interference could cause danger to the patient or others. Additionally, this experiment is important as it attempts to better represent the current state of antidepressant use wherein drugs are administered for long periods and are prescribed for both males and females.

The major aim is to examine the effects three antidepressants have on memory and anxiety, in both male and female rats, using the responsiveness to brightness change paradigm and modified OFT (to test emergence behaviour). Previously outlined behavioural tests involve many measures, providing substantial data for consideration when assessing emotionality and cognition. These behavioural tests also allow the drug effects on emotionality and cognition to be assessed without interference or influence from depressive symptoms. Due to disagreement in the literature regarding antidepressants effects on memory and anxiety, directional hypotheses were not made, in favour of reserving judgment until results were available.

Methods

Subjects

Subjects were 70 male and 70 female PVG/C hooded rats bred in the Animal Facility, Department of Psychology, University of Canterbury, New Zealand. On post natal day (PND) 30, pups were weaned and housed in 550 x 360 x 220mm opaque plastic cages in same sex groups of two to four. Temperature and humidity controlled colony rooms ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and rh $48\% \pm 10\%$). Rats were kept on 12 hour light/dark cycle (lights on at 0800) and tested during the light phase. All rats had access to water and food (commercial rat pellets) ad libitum until the start of the testing phase. All subjects and procedures were approved by the University of Canterbury Animal Ethics Committee (see Appendix A).

To determine the minimum number of rats required to generate statistically significant results a statistical power analysis for a 4 x 2 x 2 repeated measures factorial ANOVA design was performed. A minimum of 128 rats resulted. A total of 140 rats was decided upon to allow for adverse consequences, such as death or illness amongst the rats.

Housing and Feeding Procedures

After PND 90, rats were randomly assigned to one of three drug groups or the placebo. Drug groups and the placebo consisted of 10 males and 10 females per dosage level (only one placebo group). Rats were taken from their home cages, weighed and marked with colour, to track housing in separator cages. Rats were then placed in opaque plastic cages measuring 620mm X 400mm X 220mm which had a wire mesh separator lengthways centrally in the cage. One rat was placed in each side of the separated cage, allowing visual and olfactory (but not tactile) contact between rats. This type of housing was used to allow specific dosing as it involves separate water and food sources for each animal. Specific dosing was required to ensure each subject reached the target drug dose.

Food used during the testing phase consisted of standard food pellets, crushed using a rock crushing machine into a coarse powder. Food was then given as a paste using a mixture of one part water to two parts powder. Powdered drugs were mixed into each food bowl individually. Rats were given the food mash, without drugs, for seven days to allow adaptation to the new method of food administration, and to determine food consumption for each rat on a daily basis. After this phase, powdered drugs were combined with the food and administered to each rat for a minimum of 21 days. Behavioural tests were performed at the end of this period.

Drugs and Rationale for Doses

Three drugs were investigated in this experiment; the SSRI Fluoxetine, the SNRI Venlafaxine and the NRI Reboxetine. Two target doses of each drug were used and a placebo group (Table One). Drugs were purchased from CDC Pharmaceuticals (Christchurch, New Zealand). Drugs were crushed using a mortar and pestle to allow mixing into food mash. Rats received an individualised amount of drug each day, and a consistent amount of food to ensure each rat received the target dosage.

Table 1: Dosage levels and rat numbers for each group for each drug

	Placebo	FX Low	FX High	RX Low	RX High	VX Low	VX High	Total
Dosage	0	7 mg/Kg	18 mg/Kg	10 mg/Kg	20 mg/Kg	10 mg/Kg	20 mg/Kg	
Number of Rats	20	20	20	20	20	20	20	140

NB: Each group of 20 rats included 10 males and 10 females. (FX: Fluoxetine; RX: Reboxetine; VX: Venlafaxine.)

Target doses were chosen from dose ranges previously shown to be behaviourally effective. For fluoxetine, these were 7 and 18 mg/kg (Ichikawa, Kuroki, & Meltzer, 1998; Thompson et al., 2004; Shishkina, Kalinina, & Dygalo, 2007; Holick, Lee, Hen, & Dulawa, 2008; Wang, et al., 2008). For reboxetine (Harkin et al., 1999; O’Leary et al., 2007; Manier,

Shelton, & Sulser, 2002; Grandoso, Pineda, & Ugedo, 2004) and venlafaxine (Sartori, Burnet, Sharp, & Singewald, 2004; Sağlam, Uzbay, Kayir, Çelik, & Beyazyürek, 2004) the doses were 10 and 20 mg/kg.

Apparatus and Behavioural Measures

Apparatus used were the responsiveness to brightness change paradigm to test short term memory and a combined open field box and emergence test to examine emotionality.

Responsiveness to brightness change.

This test was used to examine short term memory and curiosity behaviours (Hughes & Maginnity, 2007). The Y maze consisted of two 450 mm long arms with a 150 mm long stem. The maze was 100 mm wide and 140 mm high with a hinged transparent Perspex lid covering the arms and stem. A removable black or white insert (floor and two side walls) was placed in each arm which covered the width, height and last 400 mm of length of each arm. The Y-maze has two stages, the acquisition stage and the retention stage. Between stages the rats were placed back into home cages. Between every trial the Y-maze, inserts, lid, the light/dark box and the open field were cleaned with a 2- 4% solution of “Power Quat Blue” to remove odours from the apparatus.

Acquisition trial.

Rats were given free access to the Y-maze with one black arm and one white arm for five minutes. Rats were then removed from the apparatus placed in the home cage while the arm inserts were changed to clean black ones. The side which had the white insert was alternated to control for direction effects.

Retention trial.

Rats were placed in the Y-maze and allowed to freely explore for exactly three minutes. The first arm entered by the bulk of the rat was recorded and every three seconds it was noted

on data sheets whether the rat was in the left arm, right arm or stem. Three second intervals were signalled by an auditory “beeper.”

The Emergence test.

This test was used to examine the behaviours of anxiety and impulsivity (Hascoët, Bourin, & Nic Dhonnchadha, 2001).

Emergence latency from the dark compartment into the light open field was assessed in a combined emergence/open field apparatus consisting of a 600 mm x 200 mm x 300 mm compartment separated from the open field by a wooden partition. Rats were able to move between the two sides through a 100 mm x 100 mm opening in the centre of the partition that could be closed with a movable horizontal slide. The dark side was covered by a hinged wooden lid, and the light side was open. Rats were placed in the dark side for approximately 60s with the separating slide closed. Then, the slide was opened and the latency for the rat to emerge into the light side far enough for the slide to be shut behind it was measured.

Open field.

This test was used to examine the behaviours of activity and emotionality (Archer, 1973). The open field consisted of a 600 x 600mm wooden open field with Perspex walls 250mm high. The floor comprised white Perspex divided into 16 numbered squares by a grid of intersecting lines; the squares were all the same size and numbered 1-16. Once a rat emerged from the dark box, it was noted every three seconds which square the rat was located in using the auditory “beeper”, for five minutes. Also noted was if the rat was rearing up on its hind legs (R) or grooming (G). At the end of the five minutes the number of faecal boluses left in the apparatus (defecation) was counted. Subjects were alternated between performing the responsiveness to brightness change paradigm or the combined emergence/open field paradigm first to reduce the possibility of order effects.

Results

No differences were found for rearing or centre square occupancy in any drug group (all p 's > 0.05). Likewise there were no differences in the Y-Maze when considering total arm entries for any group (all p 's > 0.05).

The figures for the following results are shown at the end of this section (p.35).

Emergence test

Drug effects are shown in figure 1.

Latency to emerge.

Fluoxetine. Five male (one placebo, one low dose and three high dose) and three female (one low dose and two high dose) rats that failed to emerge from the dark side were excluded from analysis. Of the remaining rats, males ($M = 153.24$, $SE = 17.88$) took significantly longer to emerge than females ($M = 88.67$, $SE = 11.82$; $F(1, 51) = 11.95$, $p = 0.001$). As illustrated in Figure 1, low dose rats took significantly longer to emerge from the darkened start-box than either placebo or high dose animals (main effect of dose: $F(2, 51) = 6.26$, $p = 0.039$). Males and females did not differ at different dosage levels ($F(2, 51) = 1.54$, $p = 0.2244$). An examination of Figure 1 shows that the emergence time increased compared with placebo for the low dose group and then dropped back down for the high group.

Reboxetine. Three males (One placebo and two high dose) and one female (low dose) that failed to emerge into the light side were excluded from this analysis. Males ($M = 128.59$, $SE = 17.05$) and females ($M = 86.97$, $SE = 15.05$) did not differ in emergence latency and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Venlafaxine. Seven male (one placebo, two low dose and four high dose) and six female rats (five low dose and one high dose) failed to emerge and were excluded from this analysis. For the remaining rats, males ($M = 113.61$, $SE = 22.23$) and females ($M = 81.38$, $SE = 13.49$) did not differ in emergence latency. High dose rats took longer to emerge than low dose of placebo rats ($F(2, 47) = 3.98$, $p = 0.02$). Again, there were no differences between the sexes at different dosages (All p 's > 0.05).

Overall, there was a greater emergence latency for low dose fluoxetine and reduced emergence latency for low dose venlafaxine.

Open Field

The OFT was used to measure emotionality and activity. As noted previously emotionality is analogous to anxiety. Seven behavioural measures were recorded in the OFT.

Ambulation.

Drug effects for this measure are displayed in Figure 2.

Fluoxetine. Males ($M = 12.43$, $SE = 2.09$) and females ($M = 14.5$, $SE = 2.06$) did not differ in ambulation, nor was there a difference between the dose levels ($F(2, 59) = 1.62$, $p = 0.2081$). However, males and females did differ between the dose levels ($F(2, 59) = 3.87$, $p = 0.0269$). As shown in Figure 2, low dose female rats displayed a significantly lower level of ambulation than placebo females. Low dose females were also significantly lower on this measure than high dose females. High dose females were not significantly different from placebo. Examining Figure 2A shows a similar pattern to that seen in the emergence test and suggests an anxiogenic effect occurring at the lower dose which declined as the dose increased

Reboxetine. Males ($M = 13.7$, $SE = 1.99$) and females ($M = 15.47$, $SE = 2.02$) did not differ in ambulation and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Venlafaxine. Males ($M = 11.6$, $SE = 2.10$) and females ($M = 15.4$, $SE = 2.07$) did not differ in ambulation and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Overall, fluoxetine was the only drug to show differences from placebo in ambulation and this was only for females. Low dose females displayed lower ambulation than high dose or placebo.

Rearing.

Drug effects for rearing can be seen in Figure 3.

Fluoxetine. Males ($M = 6.43$, $SE = 1.42$) and females ($M = 3.97$, $SE = 0.94$) did not differ in rearing and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Reboxetine. Males ($M = 8.63$, $SE = 1.36$) and females ($M = 5.4$, $SE = 0.96$) did not differ in rearing and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Venlafaxine. Males ($M = 7.53$, $SE = 1.44$) and females ($M = 4.83$, $SE = 2.43$) did not differ in rearing and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Overall, no differences from placebo were seen between males and females or for any drug level.

Grooming.

Drug effects for this response are outlined in in Figure 4.

Fluoxetine. Females (13.47 ± 2.49) groomed more frequently than males ($M = 3.57$, $SE = 1.09$, $F(1, 59) = 13.67$, $p = 0.0005$). Males and females did not differ at different dosage levels, nor were there difference between the drug groups themselves (All p 's > 0.05).

Reboxetine. Males ($M = 4.8$, $SE = 1.20$) and females ($M = 6.73$, $SE = 1.89$) did not differ in grooming, nor did the dose levels differ ($F(2, 59) = 1.16$, $p = 0.3208$). However, males and females did differ within the dosages ($F(2, 59) = 6.06$, $p = 0.0042$, see Figure 4B). This difference is due to high and low dose females grooming less than placebo.

Venlafaxine. Males ($M = 4.97$, $SE = 1.74$) and females ($M = 8.2$, $SE = 2.03$) did not differ in grooming nor were there differences between the drug groups themselves ($F(2, 59) = 0.9$, $p = 0.4112$). Again, males and females did differ within the dosages ($F(2, 59) = 3.68$, $p = 0.0318$, Figure 4C). This is due to low dose male rats grooming more than placebo. Interestingly, male placebo rats (1.9 ± 1.02) groomed significantly less often than female placebo rats (13.9 ± 4.38).

Overall, reboxetine females groomed less than placebo, while venlafaxine low dose males groomed more.

Faecal Boluses.

See Figure 5 for the drug effects on this measure.

Fluoxetine. Low dose rats produced more faecal boluses than placebo ($F(2, 59) = 3.98$, $p = 0.02$). Examining Figure 5 shows a similar pattern to that seen in the emergence test and suggests an anxiogenic effect occurring at the lower dose which declines as the dose increases. Males ($M = 1.53$, $SE = 0.37$) and females ($M = 0.87$, $SE = 0.24$) did not differ in

number of boluses produces and there were no differences between the sexes at different doses ($F(2, 59) = 1.71, p = 0.19$)

Reboxetine. Males ($M = 0.30, SE = 0.13$) and females ($M = 0.47, SE = 0.21$) did not differ in number of boluses and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Venlafaxine. Males ($M = 1.00, SE = 0.26$) defecated more than females ($M = 0.43, SE = 0.14$). There was no difference between the doses ($F(2, 59) = 1.53, p = 0.2257$). However, low dose males ($M = 1.4, SE = 0.56$) produced more boluses than low dose females ($M = 0.1, SE = 0.10; F(2, 59) = 3.22, p = 0.0479$).

Occupancy of centre squares.

The main effects for this measure are outlined in Figure 6.

Fluoxetine. Males ($M = 1.5, SE = 0.87$) and females ($M = 0.93, SE = 0.24$) did not differ in centre square occupancy and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Reboxetine. Males ($M = 1.27, SE = 0.49$) and females ($M = 0.93, SE = 0.24$) did not differ in centre square occupancy and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Venlafaxine. Males ($M = 1.27, SE = 0.42$) and females ($M = 1.13, SE = 0.26$) did not differ in centre square occupancy and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Occupancy of corner squares.

See Figure 7 for the effects of the drugs on this measure.

Fluoxetine. Males ($M = 77.07$, $SE = 4.08$) and females ($M = 71.80$, $SE = 3.83$) did not differ in corner square occupancy and did not differ between sex at different doses ($F(2, 59) = 0.36$, $p = 0.6996$). Low dose rats occupied corner squares less than placebo ($F(2, 59) = 3.79$, $p = 0.02$)

Reboxetine. Males ($M = 71.87$, $SE = 5.24$) and females ($M = 82.53$, $SE = 2.29$) did not differ in corner square occupancy and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Venlafaxine. Males ($M = 71.87$, $SE = 5.24$) and females ($M = 82.53$, $SE = 2.29$) did not differ in corner square occupancy and there were no differences for different dosage levels ($F(2, 59) = 1.65$, $p = 0.20$). Low dose male rats occupied corner squares significantly less than placebo ($F(2, 59) = 3.46$, $p = 0.0386$).

Overall, low dose fluoxetine rats occupied corner squares less than placebo, a result which is reflected by low dose male venlafaxine rats.

Y-Maze

The Y-Maze test was used in this experiment to measure short term spatial recognition memory. There are five different measures within this paradigm.

First arm entered.

Table 2 shows the results of a chi square analysis of the rats' preferences for entering the novel arm first in the retention trial. Visual inspection of the data revealed no difference between males and females regarding responsiveness to the drugs and as such the sexes were not separated for these analyses. Two rats were removed from the analysis from the placebo

group as they failed to enter either arm. This was also done for three rats from the low and one rat from the high fluoxetine group. One rat was removed from the analysis for each of the high and low reboxetine groups. Two rats were each removed from the high and low venlafaxine groups. These rats were excluded from all of the following measures, except the total entries of both arms measure. This is due to no entries of either arm being a valid result for this measure, whereas this is not a valid result in the other measures.

As seen in table 2; placebo rats exhibited a preference for entering the novel arm first in the retention phase. With respect to the drug groups; for reboxetine and venlafaxine neither dose level displayed a preference for the novel arm in the retention phase. For fluoxetine, the low dose group did not have a preference for the novel arm in this second phase; however the high dose group did significantly prefer entering the novel arm first.

Table 2 Chi square analyses of the effects of fluoxetine, reboxetine and venlafaxine on numbers of rats that entered the novel arm first.

Placebo	Dose 1	Dose 2	X^2 (df = 2)	P
<i>Fluoxetine</i>				
15/18 rats (83%)	12/17 rats (71%)	16/19 rats (84%)	1.26	>0.5
*(P = 0.008)	(P >0.1)	(P = 0.004)		
<i>Reboxetine</i>				
15/18 (83%)	12/19 rats (63%)	14/19 rats (74%)	1.92	>0.3
	(P >0.3)	(P >0.06)		
<i>Venlafaxine</i>				
15/18 (83%)	11/18 rats (61%)	13/18 rats (72%)	2.22	>0.3
	(P >0.6)	(P >0.09)		

Males (overall) = 48/62 (77%); Females = 45/66 (68%), X^2 (df = 1) = 1.38, P >0.2.

*Probability values beneath each dose level (1= low dose, 2= high dose) indicate whether or not the numbers of rats that entered the novel arm first were greater than chance expectancies of 50% (two-tailed binomial tests).

As shown in Table 2, differences between the dose groups were not significant for any of the three drugs.

Percentage of entries of the novel arm.

Effects of the drugs are outlined in Figure 8.

Fluoxetine. Males ($M = 72.22$, $SE = 6.10$) and females ($M = 69.42$, $SE = 6.28$) did not differ in percentage entries of the novel arm, nor was there a difference between the doses ($F(2, 53) = 1.12$, $p = 0.3332$). Male rats made fewer entries of the novel arm than placebo (that also significantly preferred the novel arm, one-sample t-test; $F(2, 53) = 3.3$, $p = 0.04$).

Reboxetine. Males ($M = 75.37$, $SE = 5.38$) and females ($M = 61.42$, $SE = 6.19$) did not differ in percentage entries of the novel arm and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Venlafaxine. Males ($M = 65.34$, $SE = 6.23$) and females ($M = 64.18$, $SE = 6.35$) did not differ in their entries of the novel arm. A difference was seen between the doses ($F(2, 52) = 5.21$, $p = 0.091$) and this is reflected in male rats entering the novel arm less than placebo (that significantly preferred the novel arm; $F(2, 52) = 4.73$, $p = 0.0134$).

Overall, male fluoxetine and reboxetine rats made fewer entries of the novel arm than placebo males.

Percentage of time spent in novel arm.

Effects of the drugs can be seen in Figure 9.

Fluoxetine. Females ($M = 55.36$, $SE = 7.61$) spent less time in the novel arm of the Y-maze than male rats ($M = 75.33$, $SE = 6.29$). High dose rats spent significantly less time in the novel arm than placebo ($F(2, 53) = 4.38$, $p = 0.01$). Males and females did not differ at different dosage levels (All p 's > 0.05).

Reboxetine. Males ($M = 77.65$, $SE = 5.36$) did not differ from females ($M = 64.04$, $SE = 6.22$) in time spent in the novel arm, nor were there differences between the dosages ($F(2,$

55) = 2.27, $p = 0.11$). Low dose males spent less time in the novel arm ($F(2, 55) = 3.91$, $p = 0.02$).

Venlafaxine. Males ($M = 68.37$, $SE = 6.51$) and females ($M = 63.58$, $SE = 7.18$) did not differ in time spent in the novel arm. Males spent less time in the novel arm than placebo (Main effect of dose: $F(2, 52) = 4.4$, $p = 0.01$, sex X dose interaction: $F(2, 52) = 3.33$, $p = 0.04$). Interestingly, placebo males ($M = 98.55$, $SE = 1.45$) spent y more time in the novel arm than placebo females ($M = 64.27$, $SE = 14.05$).

Overall, fluoxetine females spent less time in the novel arm than males. This was also reflected in high dose fluoxetine, low dose reboxetine males and venlafaxine males when compared with placebo.

Total entries into both arms.

The effects of the three drugs on this measure can be seen in Figure 10A.

Fluoxetine. Males ($M = 1.93$, $SE = 0.23$) and females ($M = 2.83$, $SE = 0.53$) did not differ in total entries into both arms and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Reboxetine. Males ($M = 2.13$, $SE = 0.30$) and females ($M = 2.8$, $SE = 0.39$) did not differ in total entries into both arms and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Venlafaxine. Males ($M = 2.5$, $SE = 0.43$) and females ($M = 2.83$, $SE = 0.41$) did not differ in emergence latency and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Total time spent in both arms.

Effects of the drugs can be seen in Figure 10B.

Fluoxetine. Females ($M = 37.26$, $SE = 3.47$) spent more time in the arms of the Y-maze than males ($M = 25.63$, $SE = 3.00$, $F(1, 53) = 7.21$, $p = 0.01$). High dose rats occupied the arms of the Y-maze for less time than placebo ($F(2, 53) = 3.19$, $p = 0.0502$). The sex-dose interaction was not significant ($F(2, 53) = 0.39$, $p = 0.6761$).

Reboxetine. Males ($M = 31.73$, $SE = 3.11$) and females ($M = 27.78$, $SE = 3.10$) did not differ in emergence latency and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Venlafaxine. Males ($M = 31.65$, $SE = 3.31$) and females ($M = 31.37$, $SE = 3.00$) did not differ in emergence latency and there were no differences for different dosage levels or between sexes at different doses (All p 's > 0.05).

Overall, fluoxetine females spent more time in the Y-maze arms than males, while high dose rats occupied the arms less than placebo.

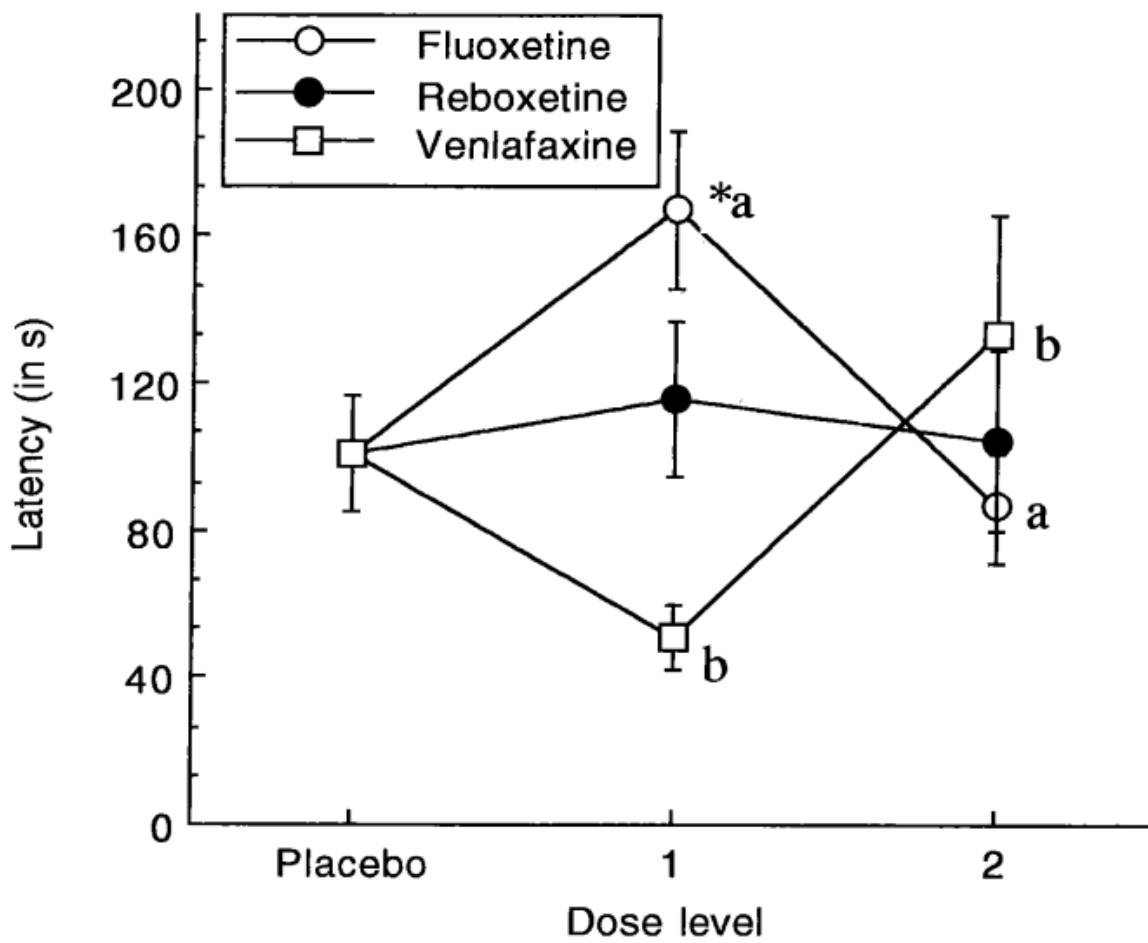


Figure 1: Mean (\pm SEM) emergence latency for both sexes combined following chronic exposure to normal diet (placebo), and two doses (1= low dose, 2= high dose) each of fluoxetine, reboxetine and venlafaxine.

*significantly different ($p < 0.05$) from placebo; ^{a,b} differences between groups with superscripts in common are significant ($p < 0.05$).

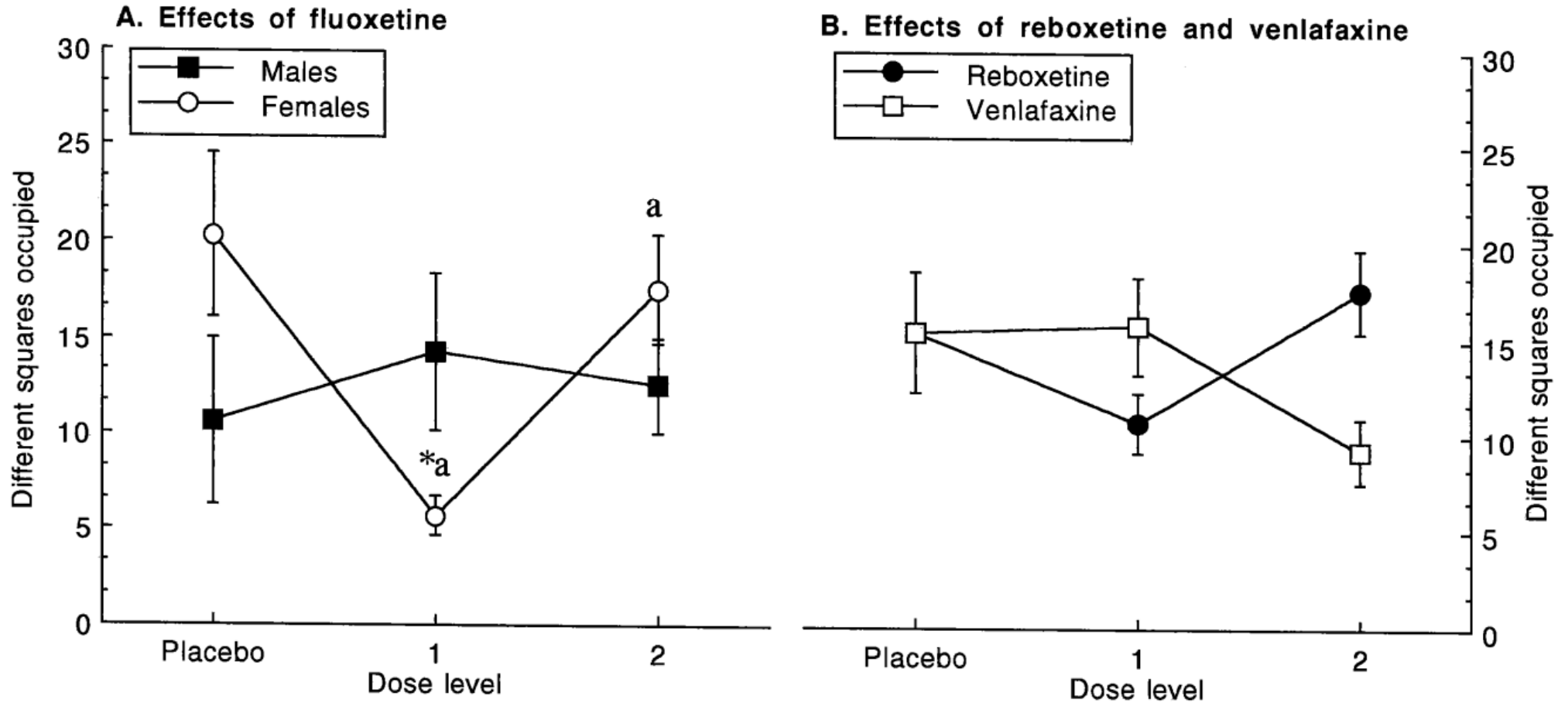


Figure 2: Mean (\pm SEM) ambulation for, **A.** males and females separately following chronic exposure to normal diet (placebo) and two doses (1= low, 2= high) of fluoxetine, and for **B.** both sexes combined following exposure to normal diet (placebo) and two doses (1= low, 2= high) each of reboxetine and venlafaxine.

*significantly different ($p < 0.05$) from placebo; ^adifference between groups with superscripts in common are significant ($p < 0.05$).

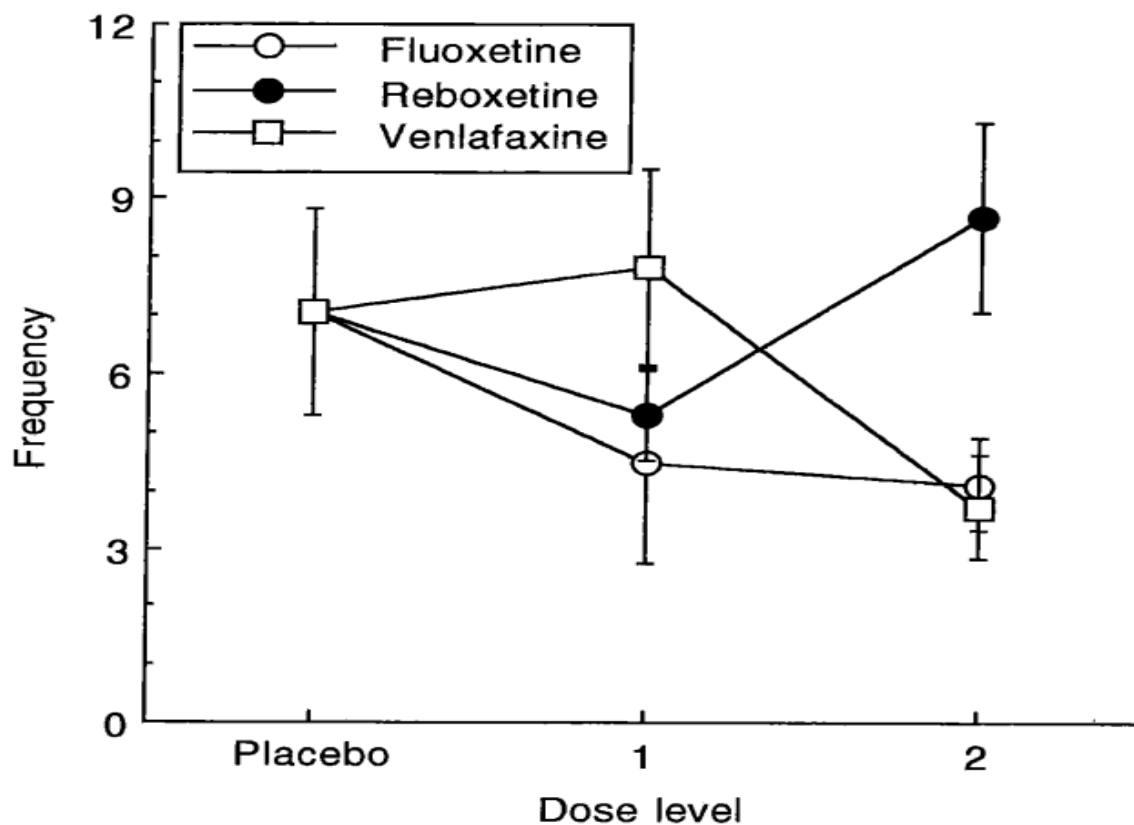


Figure 3: Mean (\pm SEM) rearing frequency for both sexes combined following chronic exposure to normal diet (placebo), and two doses (1= low, 2= high) each of fluoxetine, reboxetine and venlafaxine.

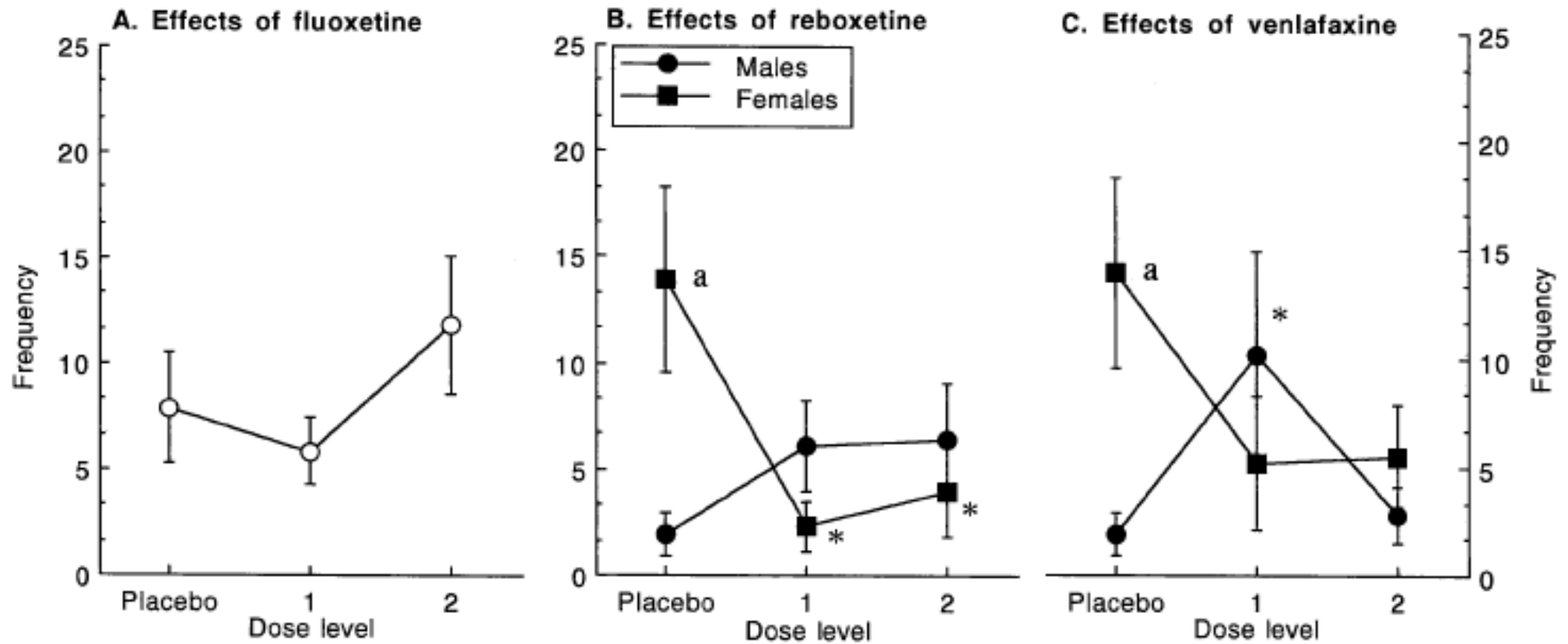


Figure 4: Mean (\pm SEM) grooming frequency for, **A.** both sexes combined following exposure to normal diet (placebo) and two doses (1= low, 2= high) of fluoxetine, and for males and females separately following chronic exposure to normal diet (placebo) and two doses (1= low, 2= high) of **B.** reboxetine, and **C.** venlafaxine.

^{*}significantly different ($p < 0.05$) from placebo. ^aSex difference significant ($p < 0.05$) for the placebo group.

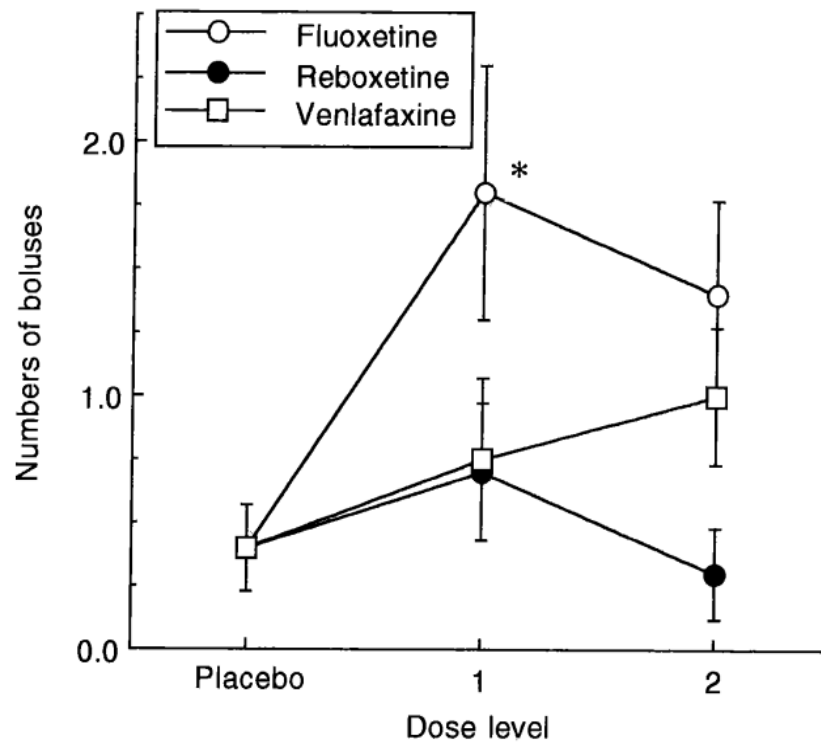


Figure 5: Mean (\pm SEM) number of faecal boluses for both sexes combined following chronic exposure to normal diet (placebo), and two doses (1= low, 2= high) each of fluoxetine, reboxetine and venlafaxine.

*significantly different ($p < 0.05$) from placebo.

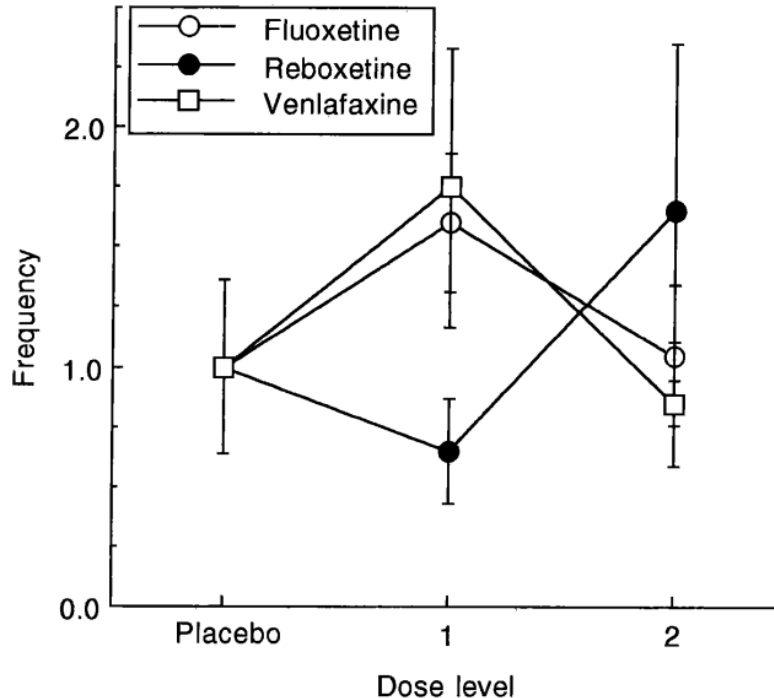


Figure 6: Mean (\pm SEM) frequency of centre squares occupancy for both sexes combined following chronic exposure to normal diet (placebo), and two doses (1= low, 2= high) each of fluoxetine, reboxetine and venlafaxine.

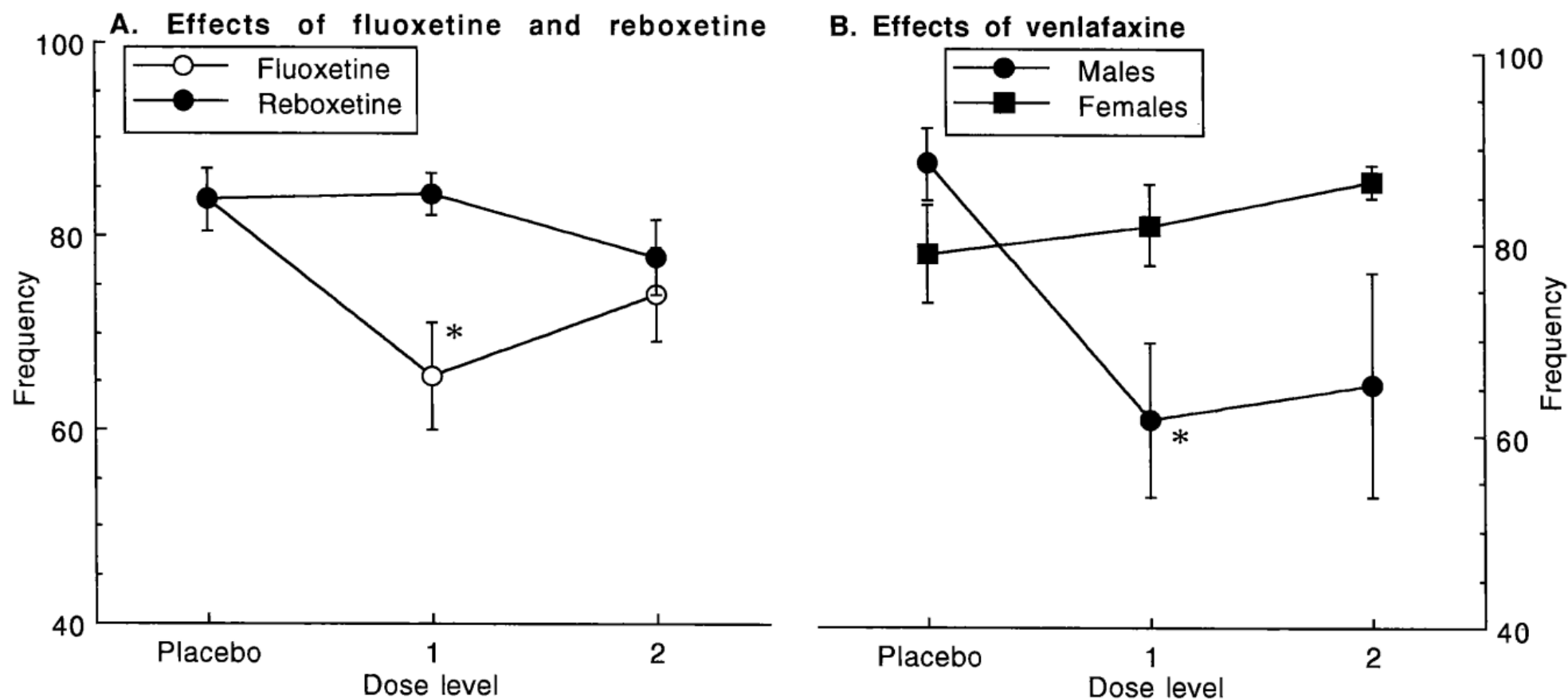


Figure 7: Mean (\pm SEM) frequency of corners occupancy for, **A.** both sexes combined following exposure to normal diet (placebo) and two doses (1= low, 2= high) each of fluoxetine and reboxetine, and **B.** for males and females separately following chronic exposure to normal diet (placebo) and two doses (1= low, 2= high) of venlafaxine.

*significantly different ($p < 0.05$) from placebo.

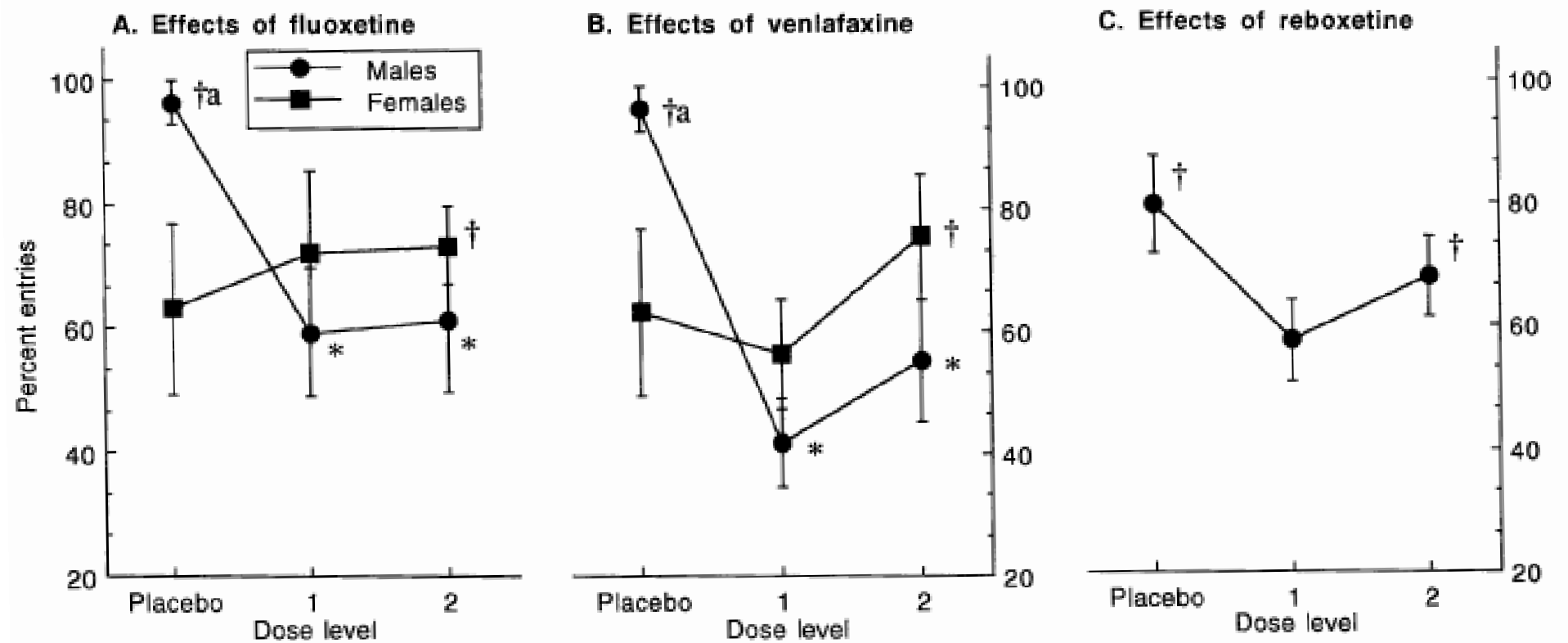


Figure 8: Mean (\pm SEM) percent entries of the novel Y-maze arm for, **A.** males and females separately following exposure to normal diet (placebo) and two doses (1= low dose, 2= high dose) each of **A.** fluoxetine, and **B.** venlafaxine, and for **C.** both sexes combined following chronic exposure to normal diet (placebo) and two doses of reboxetine.

*significantly different ($p < 0.05$) from placebo; [†]frequency significantly higher ($p < 0.05$) than a chance expectancy of 50%. ^aSex difference significant ($p < 0.05$) for the placebo group.

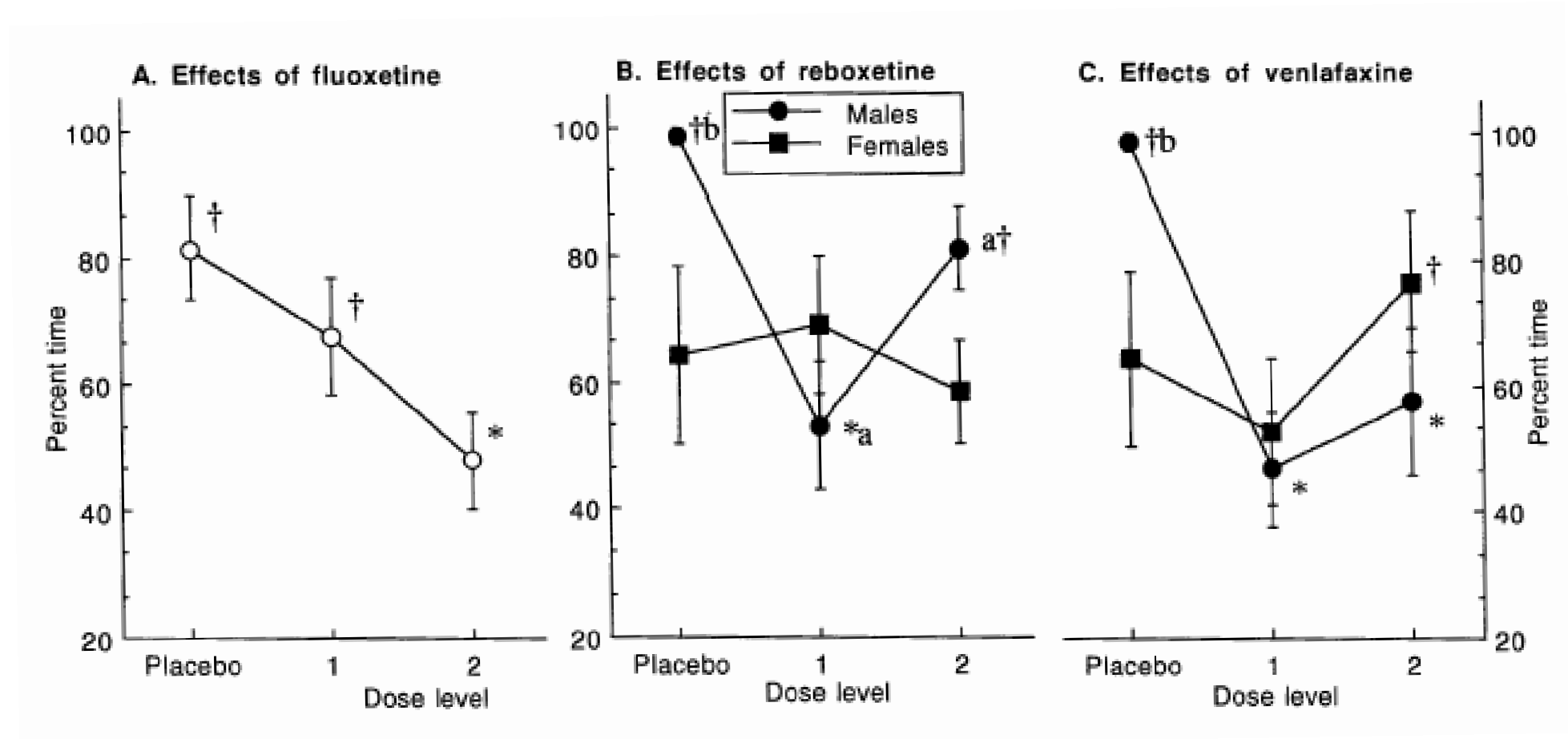


Figure 9: Mean (\pm SEM) percent time spent in the novel Y-maze arm for, **A.** both sexes combined following chronic exposure to normal diet (placebo) and two doses (1= low dose, 2= high dose) of fluoxetine, and for males and females separately following exposure to normal diet (placebo) and two doses (1= low dose, 2= high dose) each of **B.** reboxetine, and **C.** venlafaxine.

*significantly different ($p < 0.05$) from placebo; a difference between groups with superscripts in common significant ($p < 0.05$); †frequency significantly higher ($p < 0.05$) than a chance expectancy of 50%. bSex difference significant ($p < 0.05$) for the placebo group.

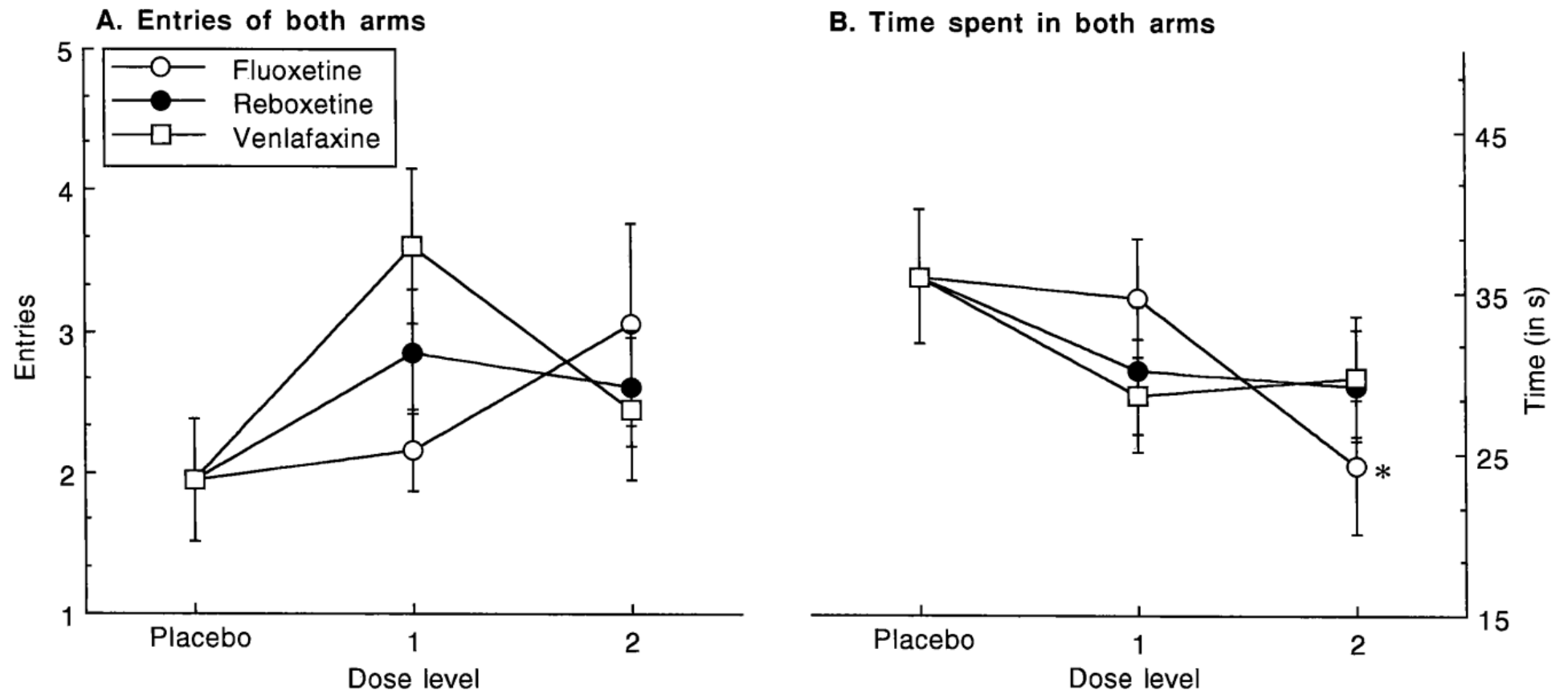


Figure 10: Mean (\pm SEM) number of, A. entries into, and B. time spent in both Y-maze arms for both sexes combined following chronic exposure to normal diet (placebo), and two doses (1= low dose, 2= high dose) each of fluoxetine, reboxetine and venlafaxine.

*significantly different ($p < 0.05$) from placebo.

Discussion

Summary of Results

For rats consuming low doses of fluoxetine, there was evidence of increased levels of anxiety from open-field responses. There was no change in anxiety at high doses of fluoxetine. Regarding memory, deficits in initial attention were seen in low dose rats due to an inability to recognise the novel Y-maze arm, but an overall preference for the novel arm indicated no deficit in overall short term memory. High dose rats were able to distinguish the novel arm initially but showed an overall preference for the familiar arm. This suggests no short term memory deficits but that habituation to novelty is present, particularly in male rats.

Rats consuming either dose of reboxetine showed no changes in anxiety. Female low dose rats had deficits in initial attention, due to a failure to recognise the novel arm. Male low dose rats not only showed these initial deficits, but also spent less time in the novel arm suggesting a substantial deficit in overall short term memory. Both male and female high dose rats showed deficits in initial attention, however not in overall short term memory.

Venlafaxine results were more complex than either fluoxetine or reboxetine. Male rats consuming low doses of venlafaxine showed an increase in anxiety but this was not seen in high dose males. This was supported by the Y-maze data and substantial neophobia. Neither group of females demonstrated anxiety effects. Deficits in short term memory were found in the y-maze in both the initial and longer term measures for males. The Y-maze results suggest there was a deficit in initial attention for high dose females but not in overall short term memory in contrast to males.

Anxiety Results:

The tests used in this experiment (OFT, emergence test and the response to brightness change paradigm) have been used extensively and have good construct validity (Hughes & Maginnity, 2007; Smythe et al., 1996, 1998; Timothy et al., 1999), and are replicable and reliable tests (Dember & Millbrook, 1956; Lukaszewska, 1978; Walk, 1960; Broadhurst, 1975; Denenberg, 1969; Hall, 1934; Royce, 1977; Walsh & Cummins, 1976; Whimbey & Denenberg, 1967a; 1967b; Ardayfio & Kim, 2006). The wealth of literature involving these tests provides assurance that the results reliably measure anxiety and memory. When considering the OFT, level of ambulation and faecal boluses are considered the most common, reliable and valid measures (Livesey & Egger, 1970; Broadhurst, 1976; Gray, 1987; Walsh & Cummins, 1976; Broadhurst, 1957; Whimbey & Denenberg, 1967b). Results from this experiment, namely that rearing and occupancy of centre squares measurements were not significant, yet anxiety was clearly present for some groups reflects this consideration.

Rats receiving low doses of fluoxetine demonstrated higher anxiety in terms of emergence, ambulation and faecal boluses., with all measures showing an inverted u-shape dose response curve. The occupancy of corner squares measure seems to contradict other anxiety findings, suggesting lower anxiety at low doses of fluoxetine. However, additional examination of the OFT data (Appendix 2) provided evidence for thigmotaxis, suggested by the large amount of time spent in peripheral squares close to the dark box exit As mentioned previously, thigmotaxis is a well validated measure of emotionality in rats (Treit & Fundytus, 1989; van der Staay, Kerbusch, & Raaijmakers, 1990). This is an interesting result to note as fluoxetine is also used as an anti-anxiety medication (Baldwin et al., 2005). An acknowledged side-effect of fluoxetine during the initial phase of administration is increased anxiety. However, in humans this tends to dissipate in two to three weeks after initial dosing

(Stokes & Holtz, 1997). As the current experiment set out to replicate the chronic use of antidepressants, initial anxiety should have been overcome. It is possible that the lower dose used did not allow a chronic level of fluoxetine to accumulate and due to this the side effect of anxiety remained. A recommended starting dose for fluoxetine in humans is 20 mg/day (Hurst & Lamb, 2000) however, rodent experiments have previously shown chronic administration of 7 mg/kg/day to be effective (Thompson et al., 2004; Shishkina et al., 2007).

In terms of future research, the anxiogenic effect of fluoxetine at lower doses (7mg/day) but not at higher doses is of key importance as fluoxetine used not only for MDD, but also for treatment of anxiety disorders. Attention should be given to the possibility that a lower dose given chronically may increase anxiety or not allow initial anxiety to dissipate, thus complicating effective treatment. It would be beneficial for this result specifically to be further investigated, especially to determine if the effect is present in humans.

Similar to fluoxetine, low dose venlafaxine (male rats only) demonstrated greater levels of anxiety, signified by higher grooming and more faecal boluses. Thigmotaxis was suggested to be a factor for this group as well (Appendix 2) supporting the increased anxiety shown by other measures. Low dose venlafaxine males also demonstrated anxiety in the responsiveness to brightness change task, spending less time in and making fewer entries of both arms compared to placebo rats. Taking these results alongside the other Y-maze results that showed the rats failed to initially identify the novel arm, spent less time in the novel arm and made significantly fewer entries of the novel arm than placebo, it is concluded that, rather than memory deficits being present, this profile of results was due to fear-related novelty avoidance.

Such an increase in anxiety is unlikely to be helpful to a depressed patient and may add to their symptoms. Whether venlafaxine is being given as an anti-anxiety agent also

needs consideration. Further animal studies of behaviour and neurological studies should be undertaken alongside human trials to determine if this effect is also present in humans.

As noted previously, there is debate regarding the motivation of open-field behaviour which could limit the interpretation of this finding (increased male anxiety). It is questioned whether increased ambulation and movement reflect low anxiety-related curiosity (Hayes, 1960) or high anxiety-related attempts to escape (Welker, 1957,1959) The current interpretation took into account all of the open-field measures rather than relying on single responses in order to ensure behaviour was more likely to reflect anxiety.

Consideration needs to be given to the strengths and limitations of the experiment related to anxiety. The feeding method in this study differs from free access to food pellets usually allowed for laboratory rats. Instead, rats were fed once a day and their food was in the form of a mash made from crushed pellets and water combined with powdered drugs. Rats had to learn to eat the mash before it became dry and inedible. This was a significant change as rats are grazing animals, consuming small amounts frequently throughout the day. A seven-day adjustment period using mash without drug was used to accustom the rats to this feeding method. However, because of its unusual nature, the method of food delivery may have affected the emotional state of the rats.

The administration of drugs via food is hampered by many procedural difficulties. These include rats receiving food they are not used to; possible change in taste of the food; inability to pinpoint exact dosages ingested by rats; and the need to separate rats to allow individual dosing. These methods were necessitated by the prohibitive cost of sourcing drugs for injection or drinking water solution. The initial seven day period of food without drug not only established the consumption levels of each rat, but also allowed rats to become familiar with this new feeding method. However, a three-week dosing period and almost complete

food consumption on most days allows a reasonable assumption that target doses were achieved within an acceptable limit.

Furthermore, to implement individualised food dosing, rats had to be physically separated. The housing used enabled visual and olfactory, but not tactile, contact between two rats. Although not ideal, this housing method was preferable to totally isolated housing. Experiments involving isolated housing list aggression and difficulty handling as some of the effects of isolation (Hatch et al., 1963). Compared to pre-testing emotional states, some rats did seem to become more aggressive when handled during the testing phase but increased aggression should not affect anxiety and memory as measured in the current experiment. This is because the tests being used have good specificity and are not greatly affected by outside influences. Other research has demonstrated that isolated housing does not affect spatial abilities (Harris et al., 2008), so this condition should also not affect Y-maze results.

Another factor to consider is the presence of the experimenter during testing, most importantly during the emergence test. The use of automated versions of the apparatus, where there is no human present during the test has often been adopted in previous research (Bilkei-Gorzo, Gyertyan, & Levay, 1998; Hascöet et al., 2001). However, this was not possible in the current study as the experimenter had to open and close the slide between the dark box and the open field. The same experimenter carried out all testing and endeavoured to remain silent, still and out of view of rats as much as possible. The removal of faeces, urine and smell from the apparatus between each trial is also important and this action is taken to reduce the effects of a previous rat's presence on the current trial. However, it is possible that this process actually increases neophobic responses because the apparatus does not have the familiarity of the home cage environment containing the odours and waste of other rats (Bourin & Hascoët, 2003; Hascoët et al., 2001). The odour of the cleaning solvent may also

be unpleasant but as the process of cleaning was the same for all rats and apparatus, any effect on anxiety was controlled for across all trials and animals.

The use of behavioural observations alone is considered limiting by some. Measurements of drug concentrations at the end of the experimental phase would enable more precise determinants of dose-response relationships and confirm that target doses were achieved. Nevertheless, there is considerable merit in establishing drug-behaviour relationships without highly precise dosing. With respect to clinical use of the drugs concerned, they are usually administered as standard doses that do not take into account slight variations in such parameters as body weight or amount of adipose tissue that may affect bodily concentrations of the drugs.

Memory Results

Several theories must be considered to interpret the responsiveness to brightness change results. Novelty preference has been used to determine short term spatial memory impairments (McReynolds, 1962). The first arm entered is also a robust measure of short term memory (Ardayfio & Kim, 2006; Walk, 1960) and nowadays is supported by percentage of entries to, and percentage of time spent in the novel arm (Hughes, 2001). Dember and Earl (1957) suggest that the first arm entered measures initial attention arousal. As the subsequent measures indicate overall preference, a deficit across all measures would indicate short term memory impairment. It is also possible that initial attention can be impaired or diverted in some way, but that overall preference and memory is not. It should also be considered that avoidance of the novel arm can be due to neophobia representing increased anxiety (Barnett, 1958).

Novelty preference was confirmed in placebo rats, supporting previous theory (McReynolds, 1962). Lower novelty preference found in female placebo rats is likely due to a

sex difference in habituation. Habituation refers to “decrements in the arousing properties of stimulus novelty” (Hughes, 1989 p. 149). Female rats habituate more quickly to changes in the responsiveness to brightness change paradigm than male (Hughes, 2001). Faster habituation suggests that females process environmental stimuli more efficiently than males and as they are processed, the novelty value of the stimuli decreases (Hughes, 1989).

High dose fluoxetine rats showed an initial preference for the novel arm, which conflicts with the finding that high dose rats spent less time in the novel arm and that males made fewer novel arm entries and spent less time in both arms. Overall, for the high dose group the first arm entered measure implies an ability to distinguish the novel arm from the familiar. However, the measures of number of entries of, and time spent in the novel arm suggest a loss of preference for the novel arm, indicative of habituation. This is also reflected in the decreased time spent in both arms, more time spent in the stem, and less ambulation indicating decreased arousal. The rate of habituation seems similar to that of female rats which is significant as faster female habituation is inherent in rats. As such, fluoxetine may be causing neurological changes which align the performance of high dose fluoxetine males with that of females. Future directions from this finding would involve examining the mechanisms behind faster female habituation and comparing how high doses of fluoxetine elicit this effect in male rats.

A proposed reason for antidepressant effects is that patient’s ability to cope with novel situations is restored. Research has shown fluoxetine increases the rate of habituation in olfactory bulbectomised rats (Mar, Spreekmeester, & Rochford, 2000). This same habituation effect likely explains the effects of high dose fluoxetine seen here. The dose given is strong enough to increase habituation to levels above normal for male rats. It is unclear what implication this has for depressed patients, as the rats in this study were not depressed. Over-habituation to novelty may plausibly leave patients with limited abilities to react in

unexpected situations, which could be dangerous if the stimulus presented is threatening, or in a situation such as driving. Further work would need to examine the high doses of fluoxetine to see if they could be influencing an inability to react to novel situations appropriately.

Male low dose reboxetine rats failed to initially recognise the novel arm and spent significantly less time in the novel arm. Combined, these results indicate short term memory deficits in low dose male reboxetine rats. High dose male venlafaxine rats displayed the same behaviours and had fewer entries of the novel arm than placebo, signifying deficits in short term memory within this group also. This memory deficit also needs further examination. If this deficit is found to be something occurring frequently, the suitability of this drug in treating depression must be re-examined, especially in light of research mentioned previously (Eyding et al., 2010) which questions reboxetines efficacy in treating depression as a whole.

Rats consuming low doses of fluoxetine demonstrate a preference for the novel arm (number of entries and time spent) but did not enter it initially, this suggests that memory may not have been affected, but initial attention was likely impaired. This was also seen in reboxetine rats (at low dose only for females) and venlafaxine females (both doses). Impairment in initial attention was found for drug groups, but not for placebo. It seems reasonable to assume that this stems from the drug action and needs to be examined further. As noted previously, no significant evidence of cognitive deficits have been described for fluoxetine or reboxetine. Most of these earlier studies concentrated on memory, psychomotor and sedative effects with little consideration given to effects on attention (E.g. Hindmarch, 1995, 1998; Ramaekers et al., 1995; Siepmann et al., 2001; Gallassi et al., 2006). Venlafaxine evidences minor deficits in memory, vigilance and attention (Ghoneim et al., 1984; Mackworth, 1950; O'Hanlon et al., 1998; Smith & Misiak, 1976; Seidel et al., 1985; Wechsler, 1981). However, these deficits were not significantly or consistently displayed.

The attention deficit finding was unexpected and is compelling. The hypothesis for this study was non-directional, and focussed on deficits in short-term memory. Such a deficit was found for two groups (low dose reboxetine males and high dose venlafaxine males), but it was wholly unexpected that a consistent deficit in attention would be found in the remaining groups. This provides initial evidence that there are attention deficits present for all three drugs, at both doses, for both sexes; a result not previously noted. This demonstrates a need for further testing of antidepressants and attention. Attention is an important part of learning, memory, and performing everyday tasks. Deficits in this area could hinder a patient's recovery as well as presenting dangers to themselves and others. Attention deficits may also provide a new explanation for previously reported memory deficits if tasks were attention dependent.

Sex differences:

Males given fluoxetine showed greater levels of anxiety than females evidenced by greater emergence latency and more faecal boluses. Although females displayed more grooming than males, not all authorities agree that this represents emotionality (e.g., Bolles, 1960; van Erp, Kruk, Meelis, & Willekens-Bramer., 1994). Sex differences found here support previous work indicating male rats have higher anxiety than females (Archer, 1973; Gray, 1971).

In the responsiveness to brightness change paradigm females habituated to novelty faster than males; evidenced by less time spent in the novel arm. This was further supported by greater percentage of time spent in both arms and more ambulation than males. This corresponds with faster habituation to novelty demonstrated previously (Russell, 1977; Hughes, 1990).

One constraint to the generalisability of these data may be the use of an animal study. Advantages of using rats are that they can be treated and tested in a controlled setting, resulting in fewer outside influences on behaviour. Adherence to experimental protocols can also be managed more thoroughly. Rats have a short life-span, so testing chronic use of drugs can be done within a feasible timeframe. However, drug effects may not apply to humans. Including both sexes improves the capability to generalise by highlighting effects that may be consistent and those which may vary – a methodological advantage here. As has been shown and discussed, males and females may respond differently to each drug and test. For example, as mentioned previously, female rats have been shown to habituate to novelty quicker than males (Russell, 1977), a finding supported by the placebo groups on two measures used in this study. Males are also quicker to learn spatial tasks than females (Cost et al., 2012). Hence, all measures from the Y-Maze were considered, not just first arm entered with measures of multiple entries and location preferences over time limiting the effect that any faster learning by males has on the result. There is ample evidence of sex differences in responsiveness to drugs other than those investigated in the present study (Hughes, 2007).

Concluding remarks

The overall aim of this experiment was to determine the effects of antidepressants on memory and anxiety in male and female rats. The results for each drug and dose were variable, but a deficit in initial attention was found for: low dose fluoxetine (male and female), low dose reboxetine males, high dose reboxetine (male and female), and venlafaxine females (both doses). Deficits in short term memory were found for males only in low doses of reboxetine or high doses of venlafaxine. No memory deficits were identified for fluoxetine. Anxiety was increased with fluoxetine and reboxetine given at low doses and venlafaxine in low doses for males. Initial anxiety is a known side-effect of antidepressants and sufficient amounts may not have accumulated to pass the threshold required for anxiety dissipation. More work regarding the effects of chronic use of antidepressants on memory and attention is needed especially in light of the surprising attention finding herein. This experiment provides some promising evidence of deficits in these areas, which could be significantly detrimental to those using these medications if the same effects occur in humans. Highlighted is the necessity for consideration regarding the benefits and consequences of long term use of antidepressants in future.

References

- Altamura, A. C., Montgomery, S. A., & Wernicke, J. F. (1988). The evidence for 20mg a day of fluoxetine as the optimal dose in the treatment of depression. *The British Journal of Psychiatry*, 153(S3), 109-112.
- American Psychiatric Association (Ed.). (2000). *Diagnostic and statistical manual of mental disorders: DSM-IV-TR®*. Arlington: American Psychiatric Publishing INC.
- American Psychiatric Association (APA). (2010). *Practice guideline for the treatment of patients with major depressive disorder. 3rd ed.* Arlington (VA)
- Anand, A., & Charney, D. S. (2000). Norepinephrine dysfunction in depression. *Journal of Clinical Psychiatry*, 61(S10),16-24.
- Anderson, G. M., Barr, C. S., Lindell, S., Durham, A. C., Shifrovich, I., & Higley, J. D. (2005). Time course of the effects of the serotonin-selective reuptake inhibitor sertraline on central and peripheral serotonin neurochemistry in the rhesus monkey. *Psychopharmacology*, 178(2-3), 339-346. doi: 10.1007/s00213-004-2011-7
- Archer, J. (1973). Tests for emotionality in rats and mice: A review. *Animal Behaviour*, 21(2), 205-235. doi:10.1016/S0003-3472(73)80065-X
- Archer, J. (1975). Rodent sex differences in emotional and related behavior. *Behavioral Biology*, 14(4), 451-479. doi:10.1016/S0091-6773(75)90636-7
- Ardayfio, P., & Kim, K. S. (2006). Anxiogenic-like effect of chronic corticosterone in the light-dark emergence task in mice. *Behavioral Neuroscience*, 120(2), 249-256. doi:10.1037/0735-7044.120.2.249
- Baldwin, D. S., Anderson, I. M., Nutt, D. J., Bandelow, B., Bond, A., Davidson, J. R., ... & Wittchen, H. U. (2005). Evidence-based guidelines for the pharmacological treatment of anxiety disorders: Recommendations from the British Association for Psychopharmacology. *Journal of Psychopharmacology*, 19(6), 567-596. doi:10.1177/0269881105059253
- Balfour, J. A. B., & Jarvis, B. (2000). Venlafaxine Extended-Release. *CNS Drugs*, 14(6), 483-503. doi: 10.2165/00023210-200014060-00006
- Ban, T. A., Gaszner, P., Aguglia, E., Batista, R., Castillo, A., Lipcsey, A., ... & Vergara, L. (1998). Clinical efficacy of reboxetine: A comparative study with desipramine, with methodological considerations. *Human Psychopharmacology: Clinical and Experimental*, 13(S1), S29-S39. doi: 10.1002/(SICI)1099-1077(199802)13:1+<S29::AID-HUP980>3.0.CO;2-D
- Barnett, S. A. (1958). Exploratory behaviour. *British Journal of Psychology*, 49(4), 289-310.

- Beasley, C. M., Masica, D. N., & Potvin, J. H. (1992). Fluoxetine: A review of receptor and functional effects and their clinical implications. *Psychopharmacology*, 107(1), 1-10. doi: 10.1007/BF02244958
- Beatty, W. W. (1979). Gonadal hormones and sex differences in nonreproductive behaviors in rodents: organizational and activational influences. *Hormones and Behavior*, 12(2), 112-163. doi:10.1016/0018-506X(79)90017-5
- Becker, A., Grecksch, G., Ruthrich, H. L., Pohle, W., Marx, B., & Matthies, H. (1992). Kindling and its consequences on learning in rats. *Behavioral and Neural Biology*, 57(1), 37-43. doi:10.1016/0163-1047(92)90735-M
- Belzung, C., & Griebel, G. (2001). Measuring normal and pathological anxiety-like behaviour in mice: A review. *Behavioural Brain Research*, 125(1), 141-149. doi:10.1016/S0166-4328(01)00291-1
- Benfield, P., Heel, R. C., & Lewis, S. P. (1986). Fluoxetine. *Drugs*, 32(6), 481-508. doi: 10.2165/00003495-198632060-00002
- Berlyne, D. E. (1960). *Conflict, arousal, and curiosity*. McGraw-Hill.
- Berzowski, H., van Moffaert, M., & Gagliano, C. A. (1997). Efficacy and tolerability of reboxetine compared with imipramine in a double-blind study in patients suffering from major depressive episodes. *European Neuropsychopharmacology*, 7(1), S37-S47. doi: 10.1016/S0924-977X(97)00418-5
- Bilkei-Gorzo, A., Gyertyan, I., & Levay, G. (1998). mCPP-induced anxiety in the light-dark box in rats – a new method for screening anxiolytic activity. *Psychopharmacology*, 136(3), 291-298.
- Bolles, R. C. (1960). Grooming behavior in the rat. *Journal of Comparative and Physiological Psychology*, 53(3), 306-310. doi:10.1037/h0045421
- Bourin, M., & Hascoët, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology*, 463(1), 55-65.
- Boyd, I. W. (1998). Venlafaxine withdrawal reactions. *The Medical Journal of Australia*, 169(2), 91.
- Broadhurst, P. L. (1957). Determinants of emotionality in the rat: I. Situational factors. *British Journal of Psychology*, 48(1), 1-12. doi:10.1111/j.2044-8295.1957.tb00594.x
- Broadhurst, P. L. (1975). The Maudsley reactive and nonreactive strains of rats: A survey. *Behavior Genetics*, 5(4), 299-319. doi:10.1007/BF01073201
- Broadhurst, P. L. (1976). The Maudsley reactive and nonreactive strains of rats: A clarification. *Behavior Genetics*, 6(3), 363-365. doi:10.1007/BF01065732

- Brown, S. L., Steinberg, R. L., & van Praag, H. M. (1994). The pathogenesis of depression: Reconsideration of neurotransmitter data. In den Boer, J. A. & Sitsen, J. M. (Eds.), *Handbook of Depression and Anxiety: A Biological Approach* (pp. 317-347). New York: Marcel Dekker.
- Brunello, N., & Racagni, G. (1998). Rationale for the development of noradrenaline reuptake inhibitors. *Human Psychopharmacology: Clinical and Experimental*, 13(S1), S13-S19.
- Burrows, G. D., Maguire, K. P., & Norman, T. R. (1998). Antidepressant efficacy and tolerability of the selective norepinephrine reuptake inhibitor reboxetine: A review. *Journal of Clinical Psychiatry*, 59(S14), 4-7.
- Burt, D. B., Zembar, M. J., & Niederehe, G. (1995). Depression and memory impairment: A meta-analysis of the association, its pattern, and specificity. *Psychological Bulletin*, 117(2), 285. doi: 10.1037/0033-2909.117.2.285
- Caldecott-Hazard, S., & Schneider, L. S. (1992). Clinical and biochemical aspects of depressive disorders: III. Treatment and controversies. *Synapse*, 10(2), 141-168. doi: 10.1002/syn.890100209
- Campbell, B. A., & Candland, D. K. (1961). Effects of prior shock on the emotionality of young rats in an open field. *Canadian Journal of Psychology*, 15(1), 1-5. doi:10.1037/h0083197
- Carlson, N. R. (2010). *Physiology of Behaviour*. Boston: Allyn and Bacon
- Castrén, E., Võikar, V., & Rantamäki, T. (2007). Role of neurotrophic factors in depression. *Current Opinion in Pharmacology*, 7(1), 18. doi: /10.1016/j.coph.2006.08.009
- Caul, W. F., & Barrett, R. J. (1973). Shuttle-box versus Y-maze avoidance: Value of multiple response measures in interpreting active-avoidance performance of rats. *Journal of Comparative and Physiological Psychology*, 84(3), 572. doi: 10.1037/h0034876
- Choleris, E., Thomas, A. W., Kavaliers, M., & Prato, F. S. (2001). A detailed ethological analysis of the mouse open field test: Effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. *Neuroscience & Biobehavioral Reviews*, 25(3), 235-260. doi:10.1016/S0149-7634(01)00011-2
- Conrad, C. D., Galea, L. A., Kuroda, Y., & McEwen, B. S. (1996). Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine treatment. *Behavioral Neuroscience*, 110(6), 1321. doi:10.1037/0735-7044.110.6.1321

- Coppen, A. (1969). Defects in monoamine metabolism and their possible importance in the pathogenesis of depressive syndromes. *Psychiatra, Neurologia, Neurochirurgia*, 72(2), 173.
- Corrigan, M. H., Denahan, A. Q., Wright, C. E., Ragual, R. J., & Evans, D. L. (2000). Comparison of pramipexole, fluoxetine, and placebo in patients with major depression. *Depression and Anxiety*, 11(2), 58-65. doi: 10.1002/(SICI)1520-6394(2000)11:2<58::AID-DA2>3.0.CO;2-H
- Cost, K. T., Williams-Yee, Z. N., Fustok, J. N., & Dohanich, G. P. (2012). Sex differences in object-in-place memory of adult rats. *Behavioral Neuroscience*, 126(3), 457-464. doi:10.1037/a0028363
- Costall, B., Jones, B. J., Kelly, M. E., Naylor, R. J., & Tomkins, D. M. (1989). Exploration of mice in a black and white test box: Validation as a model of anxiety. *Pharmacology Biochemistry and Behavior*, 32(3), 777-785. doi:10.1016/0091-3057(89)90033-6
- Crawley, J., & Goodwin, F. K. (1980). Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacology, Biochemistry and Behavior*, 13(2), 167-170. doi:10.1016/0091-3057(80)90067-2
- Croxtall, J. D., & Scott, L. J. (2010). Olanzapine/Fluoxetine. *CNS Drugs*, 24(3), 245-262.
- Cryan, J. F., Page, M. E., & Lucki, I. (2005). Differential behavioral effects of the antidepressants reboxetine, fluoxetine, and moclobemide in a modified forced swim test following chronic treatment. *Psychopharmacology*, 182(3), 335-344. doi: 10.1007/s00213-005-0093-5
- Dalery, J., & Honig, A. (2003). Fluvoxamine versus fluoxetine in major depressive episode: A double-blind randomised comparison. *Human Psychopharmacology: Clinical and Experimental*, 18(5), 379-384. doi:10.1002/hup.490
- Dallal, A., & Chouinard, G. (1998). Withdrawal and rebound symptoms associated with abrupt discontinuation of venlafaxine. *Journal of Clinical Psychopharmacology*, 18(4), 343.
- Delgado, P. L., Charney, D. S., Price, L. H., Aghajanian, G. K., Landis, H., & Heninger, G. R. (1990). Serotonin function and the mechanism of antidepressant action: Reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. *Archives of General Psychiatry*, 47(5), 411. doi: 10.1001/archpsyc.1990.0181017001100
- Delgado, P. L., & Moreno, F. A. (2000). Role of norepinephrine in depression. *The Journal of Clinical Psychiatry*, 61(S1), 5-12.

- Dellu, F., Mayo, W., Cherkaoui, J., Le Moal, M., & Simon, H. (1992). A two-trial memory task with automated recording: study in young and aged rats. *Brain Research*, 588(1), 132-139. doi:10.1016/0006-8993(92)91352-F
- Dember, W. N. (1956). Response by the rat to environmental change. *Journal of Comparative and Physiological Psychology*, 49(1), 93-95. doi:10.1037/h0045411
- Dember, W. N., & Fowler, H. (1958). Spontaneous alternation behaviour. *Psychological Bulletin*, 55(6), 412-428. doi:10.1037/h0045446
- Dember, W. N., & Millbrook, B. A. (1956). Free-choice by the rat of the greater of two brightness changes. *Psychological Reports*, 2, 465-467. doi:10.2466/PRO.2.7.465-467
- Denenberg, V. H. (1969). Open-field behavior in the rat: What does it mean? *Annals of the New York Academy of Sciences*, 159(3), 852-859. doi:10.1111/j.1749-6632.1969.tb12983.x
- Denenberg, V. H., & Grotta, L. J. (1964). Social-seeking and novelty-seeking behavior as a function of differential rearing histories. *Journal of Abnormal Psychology*, 69(4), 453-456. doi:10.1037/h0042003
- Detke, M. J., Johnson, J., & Lucki, I. (1997). Acute and chronic antidepressant drug treatment in the rat forced swimming test model of depression. *Experimental and Clinical Psychopharmacology*, 5(2), 107. doi:10.1037/1064-1297.5.2.107
- Dubini, A., Bosc, M., & Polin, V. (1997). Do noradrenaline and serotonin differentially affect social motivation and behaviour? *European Neuropsychopharmacology*, 7(1), S49-S55. doi: 10.1016/S0924-977X(97)00419-7
- Elliott, R. (1998). The neuropsychological profile in unipolar depression. *Trends in Cognitive Sciences*, 2(11), 447-454. doi:10.1016/S1364-6613(98)01235-2
- Encinas, J. M., Vaahtokari, A., & Enikolopov, G. (2006). Fluoxetine targets early progenitor cells in the adult brain. *Proceedings of the National Academy of Sciences*, 103(21), 8233-8238. doi: 10.1073/pnas.0601992103
- Entsuah, A. R., Rudolph, R. L., Hackett, D., & Miska, S. (1996). Efficacy of venlafaxine and placebo during long-term treatment of depression: A pooled analysis of relapse rates. *International Clinical Psychopharmacology*, 11(2), 137-145. doi:10.1097/00004850-199611020-00008
- Escorihuela, R. M., Fernández-Teruel, A., Gil, L., Aguilar, R., Tobeña, A., & Driscoll, P. (1999). Inbred Roman high- and low-avoidance rats: Differences in anxiety, novelty-seeking, and shuttlebox behaviors. *Physiology & Behavior*, 67(1), 19-26. doi:10.1016/S0031-9384(99)00064-5

- Eyding, D., Lelgemann, M., Grouven, U., Härter, M., Kromp, M., Kaiser, T., ... & Wieseler, B. (2010). Reboxetine for acute treatment of major depression: Systematic review and meta-analysis of published and unpublished placebo and selective serotonin reuptake inhibitor controlled trials. *BMJ (Clinical research ed.)*, *341*, c4737. doi:10.1136/bmj.c4737
- Fava, M. (2003). Symptoms of fatigue and cognitive/executive dysfunction in major depressive disorder before and after antidepressant treatment. *Journal of Clinical Psychiatry*, *64*, 30-34. Retrieved from <http://www.jclinpsychiatry.com/pcc/pccpdf/v05s08/v64s1406.pdf>
- Fava, M., & Kendler, K. S. (2000). Major depressive disorder. *Neuron*, *28*(2), 335-341. doi: 10.1016/S0896-6273(00)00112-4
- Fisher, P. L. (2007). Psychopathology of generalized anxiety disorder. *Psychiatry*, *6*(5), 171-175. doi: 10.1016/j.mppsy.2007.02.002
- Fowler, H. (1958). Response to environmental change: A positive replication. *Psychological Reports*, *4*, 506. doi:10.2466/pr0.1958.4.h.506
- Fudge, J. L., Perry, P. J., Garvey, M. J., & Kelly, M. W. (1990). A comparison of the effect of fluoxetine and trazodone on the cognitive functioning of depressed outpatients. *Journal of Affective Disorders*, *18*(4), 275-280. doi: 10.1016/0165-0327(90)90079-N
- Gallassi, R., Di Sarro, R., Morreale, A., & Amore, M. (2006). Memory impairment in patients with late-onset major depression: the effect of antidepressant therapy. *Journal of Affective Disorders*, *91*(2), 243-250. doi: 10.1016/j.jad.2006.01.018
- Ghoneim, M. M., Hinrichs, J. V., & Mewaldt, S. P. (1984). Dose-response analysis of the behavioral effects of diazepam: I. Learning and memory. *Psychopharmacology*, *82*(4), 291-295. doi:10.1007/BF00427672
- Gibbons, R. D., Hur, K., Hendricks-Brown, C., Davis, J. M., & Mann, J. J. (2012). Benefits from antidepressants: Synthesis of 6-week patient-level outcomes from double-blind placebo-controlled randomized trials of fluoxetine and venlafaxine. *Archives of General Psychiatry*, *69*(6), 572-579. doi: 10.1001/archgenpsychiatry.2011.2044
- Godsil, B. P., & Fanselow, M. S. (2004). Light stimulus change evokes an activity response in the rat. *Animal Learning & Behavior*, *32*(3), 299-310. doi:10.3758/BF03196029
- Goodwin, G. M. (1996). How do antidepressants affect serotonin receptors? The role of serotonin receptors in the therapeutic and side effect profile of the SSRIs. *Journal of Clinical Psychiatry*, *57*(4, Suppl), 9-13.

- Gorenstein, C., de Carvalho, S. C., Artes, R., Moreno, R. A., & Marcourakis, T. (2006). Cognitive performance in depressed patients after chronic use of antidepressants. *Psychopharmacology*, 185(1), 84-92. doi: 10.1007/s00213-005-0274-2
- Grandoso, L., Pineda, J., & Ugedo, L. (2004). Comparative study of the effects of desipramine and reboxetine on locus coeruleus neurons in rat brain slices. *Neuropharmacology*, 46(6), 815-823. doi:10.1016/j.neuropharm.2003.11.033
- Gray, J. A. (1971). Sex differences in emotional behaviour in mammals including man: Endocrine bases. *Acta Psychologica*, 35(1), 29-46. doi:10.1016/0001-6918(71)90029-1
- Gray, J. A. (1987). *The psychology of fear and stress* (Vol. 5). Cambridge University Press Archive.
- Hale, A. S., & Pinninti, N. R. (1995). Critical flicker fusion threshold and anticholinergic effects of chronic antidepressant treatment in remitted depressives. *Journal of Psychopharmacology*, 9(3), 258-266. doi: 10.1177/026988119500900309
- Hall, C. S. (1934). Emotional behavior in the rat: I. Defecation and urination as measures of individual differences in emotionality. *Journal of Comparative Psychology*, 18(3), 385-403. doi:10.1037/h0071444
- Hall, C. S., & Ballachey, E. L. (1932). A study of the rat's behavior in a field: A contribution to method in comparative psychology. *University of California Publications in Psychology*, 6, 1-12.
- Harkin, A., Kelly, J. P., McNamara, M., Connor, T. J., Dredge, K., Redmond, A., & Leonard, B. E. (1999). Activity and onset of action of reboxetine and effect of combination with sertraline in an animal model of depression. *European Journal of Pharmacology*, 364(2-3), 123. doi:10.1016/S0014-2999(98)00838-3
- Harris, A. P., D'Eath, R. B., & Healy, S. D. (2008). Sex differences in spatial cognition are not caused by isolation housing. *Behaviour*, 145(6), 757-778. doi:10.1163/156853908783929142
- Hascoët, M., Bourin, M., & Nic Dhonnchadha, B. Á. (2001). The mouse light-dark paradigm: A review. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 25(1), 141-166. doi:10.1016/S0278-5846(00)00151-2
- Hashemi, S., Shirazi, H. G., Mohammadi, A., Zadeh-Bagheri, G., Noorian, K., & Malekzadeh, M. (2012). Nortriptyline versus fluoxetine in the treatment of major depressive disorder: A six-month, double-blind clinical trial. *Clinical Pharmacology: Advances and Applications*, 4, 1-6. doi: 10.2147/CPAA.S23831
- Hatch, A., Wiberg, G. S., Balazs, T., & Grice, H. C. (1963). Long-term isolation stress in rats. *Science*, 142(3591), 507. doi:10.1126/science.142.3591.507

- Hayes, K. J. (1960). Exploration and fear. *Psychological Reports*, 6(1), 91-93. doi:10.2466/pr0.1960.6.1.91
- Hindmarch, I. (1995). The behavioural toxicity of the selective serotonin reuptake inhibitors. *International Clinical Psychopharmacology*, 9(S4), 13-17. doi: 10.1097/00004850-199501004-00002
- Hindmarch, I. (1998). Effect of antidepressants on cognitive and psychomotor function: The lack of effect of reboxetine. *Human Psychopharmacology: Clinical and Experimental*, 13(S1), S21-S27.
- Hirano, K., Kimura, R., Sugimoto, Y., Yamada, J., Uchida, S., Kato, Y., ... & Yamada, S. (2005). Relationship between brain serotonin transporter binding, plasma concentration and behavioural effect of selective serotonin reuptake inhibitors. *British Journal of Pharmacology*, 144(5), 695-702. doi: 10.1038/sj.bjp.0706108
- Holick, K. A., Lee, D. C., Hen, R., & Dulawa, S. C. (2008). Behavioral Effects of Chronic Fluoxetine in BALB/cJ Mice Do Not Require Adult Hippocampal Neurogenesis or the Serotonin 1A Receptor. *Neuropsychopharmacology*, 33(2), 406-417. doi:10.1038/sj.npp.1301399
- Holm, K. J., & Spencer, C. M. (1999). Reboxetine. *CNS Drugs*, 12(1), 65-83. doi: 10.2165/00023210-199912010-00006
- Horsburgh, R. J., & Hughes, R. N. (1981). Modification of novelty preferences in rats by current and prior treatment with scopolamine and methylscopolamine. *Psychopharmacology*, 73(4), 388-390. doi:10.1007/BF00426472
- Horst, W. D., & Preskorn, S. H. (1998). Mechanisms of action and clinical characteristics of three atypical antidepressants: Venlafaxine, nefazodone, bupropion. *Journal of Affective Disorders*, 51(3), 237-254. doi: 10.1016/S0165-0327(98)00222-5
- Hughes, R. N. (1987). Effects of adrenalin on novelty choices and activity in rats. *Life Sciences*, 41(1), 25-29. doi:10.1016/0024-3205(87)90552-2
- Hughes, R. N. (1989). Sex differences in spontaneous alternation and open-field behavior of hamsters: Habituation differences. *Current Psychology*, 8(2), 144-150.
- Hughes, R. N. (1990). Sex-dependent habituation to novelty in rats. *Current Psychology*, 9(3), 277-284.
- Hughes, R. N. (1997). Intrinsic exploration in animals: Motives and measurement. *Behavioural Processes*, 41(3), 213-226. doi:10.1016/S0376-6357(97)00055-7

- Hughes, R. N. (1999). Sex differences in novelty-related location preferences of hooded rats. *The Quarterly Journal of Experimental Psychology B*, 52(3), 235-252.
- Hughes, R. N. (2001). Responsiveness to brightness change in hooded rats: Effects of sex and procedure. *Behavioural processes*, 55(3), 143-155. doi:10.1016/S0376-6357(01)00177-2.
- Hughes, R. N. (2002). Sex-related glucose effects on responsiveness to brightness change in middle-aged rats. *Pharmacology Biochemistry and Behavior*, 73(3), 485-490.
- Hughes, R. N. (2004). The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. *Neuroscience & Biobehavioral Reviews*, 28(5), 497-505. doi:10.1016/j.neubiorev.2004.06.006
- Hughes, R. N. (2007). Sex dose matter: Comments on the prevalence of male-only investigations of drug effects on rodent behaviour. *Behavioural Pharmacology* 18, 583-589.
- Hughes, R., & Beveridge, I. (1987). Effects of prenatal exposure to chronic caffeine on locomotor and emotional behaviour. *Psychobiology*, 15(2), 179-185.
- Hughes, R. N., & Maginnity, M. E. (2007). Cues used by male and female hooded rats for locating a brightness change. *Behavioural Processes*, 74(1), 79-87.
- Hurst, M., & Lamb, H. M. (2000). Fluoxetine. *CNS drugs*, 14(1), 51-80.
- Hyman, S. E., & Nestler, E. J. (1993). *The Molecular Foundations of Psychiatry*. Washington D. C: American Psychiatric Press Inc.
- Hyttel, J. (1994). Pharmacological characterization of selective serotonin reuptake inhibitors (SSRIs). *International Clinical Psychopharmacology*, 9, 19-26. doi: 10.1097/00004850-199403001-00004
- Ichikawa, J., Kuroki, T., & Meltzer, H. Y. (1998). Differential effects of chronic imipramine and fluoxetine on basal and amphetamine-induced extracellular dopamine levels in rat nucleus accumbens. *European Journal of Pharmacology*, 350(2-3), 159-164. doi:10.1016/S0014-2999(98)00247-7
- Ivinskis, A. (1968). The reliability of behavioural measures obtained in the open-field. *Australian Journal of Psychology*, 20(3), 173-177. doi:10.1080/00049536808255754
- Ivinskis, A. (1969). *A Study of Reliability, Validity and Factor Structure of Open-field Measures* (Unpublished doctoral dissertation). University of Newcastle. Australia

- Jacobson, N., & Weiber, R. (1997). Possible delayed venlafaxine withdrawal reaction: Two case reports. *European Psychiatry: The Journal of the Association of European Psychiatrists*, 12(7), 372. doi:10.1016/S0924-9338(97)80010-7
- Kasper, S. (1999). From symptoms to social functioning: Differential effects of antidepressant therapy. *International Clinical Psychopharmacology*, 14(1), S27-S31.
- Keltner, N. L., & Folks, D. G. (2005). *Psychotropic Drugs*. St. Louis: Elsevier Mosby.
- Kivy, P. N., Earl, R. W., & Walker, E. L. (1956). Stimulus context and satiation. *Journal of Comparative and Physiological Psychology*, 49(1), 90-92. doi:10.1037/h0040984
- Kreiss, D. S., & Lucki, I. (1995). Effects of acute and repeated administration of antidepressant drugs on extracellular levels of 5-hydroxytryptamine measured in vivo. *Journal of Pharmacology and Experimental Therapeutics*, 274(2), 866-876.
- Kvist, S. B., & Selander, R. K. (1992). A qualitative aspect of learning-sensitive open field ambulation in mice. *Scandinavian Journal of Psychology*, 33(2), 97. doi:10.1111/j.1467-9450.1992.tb00889.x
- Lamberty, Y., & Gower, A. J. (1992). Age-related changes in spontaneous behavior and learning in NMRI mice from middle to old age. *Physiology & Behavior*, 51(1), 81-88. doi:10.1016/0031-9384(92)90206-H
- Latimer, P. R., Ravindran, A. V., Bernatchez, J. P., Fournier, J. P., Gojer, J. A., Barratt, K., & Butters, J. (1996). A six month comparison of toleration and efficacy of sertraline and fluoxetine treatment of major depression. *European Neuropsychopharmacology*, 6(1003), 124. doi: 10.1016/0924-977X(96)87876-X
- Lee, S. I., & Keltner, N. L., (2006). Biological Perspectives. *Perspectives in Psychiatric Care*, 42(2), 144-148. doi: 10.1111/j.1744-6163.2006.00064.x
- Lejoyeux, M., & Adès, J. (1997). Antidepressant discontinuation: A review of the literature. *The Journal of Clinical Psychiatry*, 58(S7), 11-16.
- Leppänen, P. K., Ewalds-Kvist, S. B. M., & Selander, R. K. (2005). Mice selectively bred for open-field thigmotaxis: life span and stability of the selection trait. *The Journal of General Psychology*, 132(2), 187-204. doi:10.3200/GENP.132.2.187-204
- Liebsch, G., Montkowski, A., Holsboer, F., & Landgraf, R. (1998). Behavioural profiles of two Wistar rat lines selectively bred for high or low anxiety-related behaviour. *Behavioural Brain Research*, 94(2), 301-310. doi:10.1016/S0166-4328(97)00198-8
- Lister, R. G. (1990). Ethologically-based animal models of anxiety disorders. *Pharmacology & Therapeutics*, 46(3), 321-340. doi:10.1016/0163-7258(90)90021-S

- Livesey, P. J., & Egger, G. J. (1970). Age as a factor in open-field responsiveness in the white rat. *Journal of Comparative and Physiological Psychology*, 73(1), 93-99. doi:10.1037/h0030018
- Louie, A. K., Lannon, R. A., Kirsch, M. A., & Lewis, T. B. (1996). Venlafaxine withdrawal reactions. *The American Journal of Psychiatry*, 153(12).
- Lukaszewska, I. (1978). The effects of exposure time and retention interval on response to environmental change in rats. *Acta Neurobiologiae Experimentalis*, 38(6), 323-331.
- Macbeth, R., & Rajagopalan, M. (1998). Venlafaxine withdrawal syndrome. *Australian and New Zealand Journal of Medicine*, 28(2), 218. doi:10.1111/j.1445-5994.1998.tb02977.x
- Mackworth, N. H. (1950). Researches on the measurement of human performance. *Medical Research Council Special Report Series*, 268.
- Madsen, T. M., Treschow, A., Bengzon, J., Bolwig, T. G., Lindvall, O., & Tingström, A. (2000). Increased neurogenesis in a model of electroconvulsive therapy. *Biological Psychiatry*, 47(12), 1043-1049. doi: 10.1016/S0006-3223(00)00228-6
- Malberg, J. E., Eisch, A. J., Nestler, E. J., & Duman, R. S. (2000). Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *The Journal of Neuroscience*, 20(24), 9104-9110. Retrieved from <http://www3.utsouthwestern.edu/eisch/Publications/malberg%202000.pdf>
- Manier, D. H., Shelton, R. C., & Sulser, F. (2002). Noradrenergic antidepressants: Does chronic treatment increase or decrease nuclear CREB-P? *Journal of Neural Transmission*, 109(1), 91-99. doi:10.1007/s702-002-8239-6
- Mar, A., Spreekmeester, E., & Rochford, J. (2000). Antidepressants preferentially enhance habituation to novelty in the olfactory bulbectomized rat. *Psychopharmacology*, 150(1), 52-60.
- Markowska, A., & Lukaszewska, I. (1981). Response to stimulus change following observation or exploration by the rat: differential effects of hippocampal damages. *Acta Neurobiologiae Experimentalis*, 41(4), 325.
- Massana, J. (1998). Reboxetine versus fluoxetine: An overview of efficacy and tolerability. *The Journal of Clinical Psychiatry*, 59(S14), 8-10.
- Massana, J., Möller, H. J., Burrows, G. D., & Montenegro, R. M. (1999). Reboxetine: A double-blind comparison with fluoxetine in major depressive disorder. *International Clinical Psychopharmacology*, 14(2), 73-80.

- McReynolds, P. (1962). Exploratory behavior: A theoretical interpretation. *Psychological Reports*, 11(2), 311-318. doi:10.2466/pr0.1962.11.2.311
- Mechan, A. O., Moran, P. M., Elliott, M. J., Young, A. M., Joseph, M. H., & Green, R. A. (2002). A study of the effect of a single neurotoxic dose of 3, 4-methylenedioxymethamphetamine (MDMA;" ecstasy") on the subsequent long-term behaviour of rats in the plus maze and open field. *Psychopharmacology*, 159(2), 167-175. doi:10.1007/s002130100900
- Melloni, P., Carniel, G., Della Torre, A., Bonsignori, A., Buonamici, M., Pozzi, O., ... & Rossi, A. C. (1984). Potential antidepressant agents. α -Aryloxy-benzyl derivatives of ethanolamine and morpholine. *European Journal of Medicinal Chemistry*, 19(3), 235-242.
- Millan, M. J. (2006). Multi-target strategies for the improved treatment of depressive states: Conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacology & Therapeutics*, 110(2), 135-370. doi: 10.1016/j.pharmthera.2005.11.006
- Millan, M. J., Lejeune, F., & Gobert, A. (2000). Reciprocal autoreceptor and heteroreceptor control of serotonergic, dopaminergic and noradrenergic transmission in the frontal cortex: relevance to the actions of antidepressant agents. *Journal of Psychopharmacology*, 14(2), 114-138. doi: 10.1177/026988110001400202
- Misslin, R., & Ropartz, P. (1981). Effects of methamphetamine on novelty-seeking behaviour by mice. *Psychopharmacology*, 75(1), 39-43. doi:10.1007/BF00433499
- Moody, T. W., Merali, Z., & Crawley, J. N. (1988). The effects of anxiolytics and other agents on rat grooming behavior. *Annals of the New York Academy of Sciences*, 525, 281-290. doi:10.1111/j.1749-6632.1988.tb38613.x
- Montgomery, S. A., Dufour, H., Brion, S., Gailledreau, J., Laqueille, X., Ferrey, G., ... & Danion, J. M. (1988). The prophylactic efficacy of fluoxetine in unipolar depression. *The British Journal of Psychiatry*. 157(S3), 69-76.
- Montgomery, S. A., & Schatzberg, A. F. (1998). Introduction. Reboxetine: A new selective antidepressant for the treatment of depression. *The Journal of Clinical Psychiatry*, 59(S14), 3.
- Mucci, M. (1997). Reboxetine: A review of antidepressant tolerability. *Journal of Psychopharmacology*, 11(4), S33.
- Munn, N. L. (1950). *Handbook of Psychological Research on the Rat: An Introduction to Animal Psychology*. Oxford: Houghton Mifflin.

- Muth, E. A., Haskins, J. T., Moyer, J. A., Husbands, G. E., Nielsen, S. T., & Sigg, E. B. (1986). Antidepressant biochemical profile of the novel bicyclic compound Wy-45,030, an ethyl cyclohexanol derivative. *Biochemical Pharmacology*, 35(24), 4493-4497. doi: 10.1016/0006-2952(86)90769-0
- Nakagawa, S., Kim, J. E., Lee, R., Malberg, J. E., Chen, J., Steffen, C., ... & Duman, R. S. (2002). Regulation of neurogenesis in adult mouse hippocampus by cAMP and the cAMP response element-binding protein. *The Journal of Neuroscience*, 22(9), 3673-3682. Retrieved from <http://www.jneurosci.org/content/22/9/3673.full.pdf+html>
- Nemeroff, C. B., & Amchin, J. (1998). Placebo-controlled trial of the efficacy and tolerability of venlafaxine and fluoxetine in outpatients with major depression. *European Neuropsychopharmacology*, 8(1002), S157. doi: 10.1016/S0924-977X(98)80185-5
- O'Hanlon, J. F., Robbe, H. W., Vermeeren, A., van Leeuwen, C., & Danjou, P. E. (1998). Venlafaxine's effects on healthy volunteers' driving, psychomotor, and vigilance performance during 15-day fixed and incremental dosing regimens. *Journal of Clinical Psychopharmacology*, 18(3), 212-221. doi:10.1097/00004714-199806000-00006
- O'Leary, O. F., Bechtholt, A. J., Crowley, J. J., Hill, T. E., Page, M. E., & Lucki, I. (2007). Depletion of serotonin and catecholamines block the acute behavioral response to different classes of antidepressant drugs in the mouse tail suspension test. *Psychopharmacology*, 192(3), 357-371. doi:10.1007/s00213-007-0728-9
- Paré, W. P., Tejani-Butt, S., & Kluczynski, J. (2001). The emergence test: Effects of psychotropic drugs on neophobic disposition in Wistar Kyoto (WKY) and Sprague Dawley rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 25(8), 1615-1628. doi:10.1016/S0278-5846(01)00204-4
- Parker, G., & Blennerhassett, J. (1998). Withdrawal reactions associated with venlafaxine. *Australasian Psychiatry*, 32(2), 291-294. doi:10.3109/00048679809062742
- Poucet, B., & Buhot, M. C. (1989). Scopolamine impairs response-to-change based on distal cues in the rat. *Physiology & Behavior*, 46(3), 355-359. doi:10.1016/0031-9384(89)90003-6
- Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*, 463(1), 3-33. doi:10.1016/S0014-2999(03)01272-X
- Raby, W. N. (1998). Treatment of venlafaxine discontinuation symptoms with ondansetron. *The Journal of Clinical Psychiatry*, 59(11), 621.

- Ramaekers, J. G., Muntjewerff, N. D., & O'Hanlon, J. F. (1995). A comparative study of acute and subchronic effects of dothiepin, fluoxetine and placebo on psychomotor and actual driving performance. *British Journal of Clinical Pharmacology*, 39(4), 397-404. doi: 10.1111/j.1365-2125.1995.tb04468.x
- Ramos, A., & Mormède, P. (1997). Stress and emotionality: A multidimensional and genetic approach. *Neuroscience & Biobehavioral Reviews*, 22(1), 33-57. doi:10.1016/S0149-7634(97)00001-8
- Rantamäki, T., Hendolin, P., Kankaanpää, A., Mijatovic, J., Piepponen, P., Domenici, E., ... & Castrén, E. (2007). Pharmacologically diverse antidepressants rapidly activate brain-derived neurotrophic factor receptor TrkB and induce phospholipase-C γ signaling pathways in mouse brain. *Neuropsychopharmacology*, 32(10), 2152-2162. doi: 10.1038/sj.npp.1301345
- Redrobe, J. P., Bourin, M., Colombel, M. C., & Baker, G. B. (1998). Dose-dependent noradrenergic and serotonergic properties of venlafaxine in animal models indicative of antidepressant activity. *Psychopharmacology*, 138(1), 1-8. doi: 10.1007/s002130050638
- Ricci, M., Amore, M., & Papalini, A. (1998). Venlafaxine withdrawal syndrome. *European Neuropsychopharmacology*, 8(S2), S196-S197. doi:10.1016/S0924-977X(98)80295-2
- Richman, C. L., Dember, W. N., & Kim, P. (1986). Spontaneous alternation behavior in animals: A review. *Current Psychology*, 5(4), 358-391. doi:10.1007/BF02686603
- Riva, M. A., Brunello, N., Rovescalli, A. C., Galimberti, R., Carfagna, N., Carminati, P., ... & Racagni, G. A. (1989). Effect of reboxetine, a new antidepressant drug, on the central noradrenergic system: Behavioural and biochemical studies. *Journal of Drug Development*, 1(4), 243-253. Retrieved from <http://hdl.handle.net/2434/185853>
- Rivas-Vazquez, R. A. (2001). Antidepressants as first-line agents in the current pharmacotherapy of anxiety disorders. *Professional Psychology: Research and Practice*, 32(1), 101. doi: 10.1037/0735-7028.32.1.101
- Rogóz, Z., Dziedzicka-Wasylewska, M., Margas, W., Maj, J. (1998). Some effects of the new antidepressant venlafaxine administered repeatedly [abstract]. *European Neuropsychopharmacology*, 8(S1), 33. doi:10.1007/s007020050151
- Rosen, J., & Schulkin, J. (1998). From normal fear to pathological anxiety. *Psychological Review*, 105(2), 325-350. doi: 10.1037/0033-295X.105.2.325
- Rossi, A., Barraco, A., & Donda, P. (2004). Fluoxetine: A review on evidence based medicine. *Annals of General Psychiatry*, 3(1), 2. Retrieved from <http://www.general-hospital-psychiatry.com/content/3/1/2/>

- Royce, J. R. (1977). On the construct validity of open-field measures. *Psychological Bulletin*, 84(6), 1098-1106. doi:10.1037/0033-2909.84.6.1098
- Russell, P. A. (1977). Sex differences in rats' stationary exploration as a function of stimulus and environmental novelty. *Animal Learning & Behavior*, 5(3), 297-302. doi:10.3758/BF03209243
- Rutter, J. J., Gundlach, C., & Auerbach, S. B. (1994). Increase in extracellular serotonin produced by uptake inhibitors is enhanced after chronic treatment with fluoxetine. *Neuroscience Letters*, 171(1), 183-186. doi: 10.1016/0304-3940(94)90635-1
- Sağlam, E., Uzbay, I. T., Kayir, H., Çelik, T., & Beyazyürek, M. (2004). Effects of venlafaxine on ethanol withdrawal syndrome in rats. *Fundamental & Clinical Pharmacology*, 18(6), 693-698. doi:10.1111/j.1472-8206.2004.00281.x
- Saletu, B., Grunberger, J., Anderer, P., Linzmayer, L., Semlitsch, H. V., & Magni, G. (1992). Pharmacodynamics of venlafaxine evaluated by EEG brain mapping, psychometry and psychophysiology. *British Journal of Clinical Pharmacology*, 33(6), 589-601. doi:10.1111/j.1365-2125.1992.tb04087.x
- Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., ... & Hen, R. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science Signaling*, 301(5634), 805. Retrieved from <http://www.jstor.org/stable/3834929>
- Sartori, S. B., Burnet, P. W. J., Sharp, T., & Singewald, N. (2004). Evaluation of the effect of chronic antidepressant treatment on neurokinin-1 receptor expression in the rat brain. *Neuropharmacology*, 46(8), 1177-1183. doi:10.1016/j.neuropharm.2004.02.013,
- Sashkov, V. A., Sel'verova, N. B., Morenkov, E. D., & Ermakova, I. V. (2009). Neuroactive steroids in the brain and age and sex specificity of behavior and anxiety: An experimental study. *Human Physiology*, 35(5), 556-562. doi:10.1134/S0362119709050065
- Satinder, K. P. (1969). Effects of odour trails on open-field behavior in rat. *Psychological Reports*, 25(1), 115. doi:10.2466/pr0.1969.25.1.115
- Schatzberg, A. (2000). Clinical efficacy of reboxetine in major depression. *The Journal of Clinical Psychiatry*, 61(S10), 31-38.
- Schmitt, J. A., Kruizinga, M. J., & Riedel, W. J. (2001). Non-serotonergic pharmacological profiles and associated cognitive effects of serotonin reuptake inhibitors. *Journal of Psychopharmacology*, 15(3), 173-179. doi: 10.1177/026988110101500304

- Seidel, W. F., Cohen, S. A., Wilson, L., & Dement, W. C. (1985). Effects of alprazolam and diazepam on the daytime sleepiness of non-anxious subjects. *Psychopharmacology*, 87(2), 194-197. doi:10.1007/BF00431806
- Sherwood, N. (1995). Comparative behavioural toxicity of the selective serotonin reuptake inhibitors. *Human Psychopharmacology: Clinical and Experimental*, 10(S3), S159-S162. doi: 10.1002/hup.470100904
- Shishkina, G. T., Kalinina, T. S., & Dygalo, N. N. (2007). Up-regulation of tryptophan hydroxylase-2 mRNA in the rat brain by chronic fluoxetine treatment correlates with its antidepressant effect. *Neuroscience*, 150(2), 404-412. doi:10.1016/j.neuroscience.2007.09.017
- Shrivastava, R. K., Cohn, C., Crowder, J., Davidson, J., Dunner, D., Feighner, J., ... & Patrick, R. (1994). Long-term safety and clinical acceptability of: Venlafaxine and imipramine in outpatients: with major depression. *Journal of Clinical Psychopharmacology*, 14(5), 322-329. doi:10.1097/00004714-199410000-00006
- Siepmann, M., Mück-Weymann, M., Joraschky, P., & Kirch, W. (2001). The effects of reboxetine on autonomic and cognitive functions in healthy volunteers. *Psychopharmacology*, 157(2), 202-207. doi: 10.1007/s002130100791
- Slawecki, C. J. (2005). Comparison of anxiety-like behavior in adolescent and adult Sprague-Dawley rats. *Behavioral Neuroscience*, 119(6), 1477-1483. doi:10.1037/0735-7044.119.6.1477
- Smith, J. M., & Misiak, H. (1976). Critical flicker frequency (CFF) and psychotropic drugs in normal human subjects — a review. *Psychopharmacology*, 47(2), 175-182. doi:10.1007/BF00735818
- Smythe, J. W., Bhatnagar, S., Murphy, D., Timothy, C., & Costall, B. (1998). The effects of intrahippocampal scopolamine infusions on anxiety in rats as measured by the black-white box test. *Brain Research Bulletin*, 45(1), 89-93. doi:10.1016/S0361-9230(97)00311-0
- Smythe, J. W., Murphy, D., Bhatnagar, S., Timothy, C., & Costall, B. (1996). Muscarinic antagonists are anxiogenic in rats tested in the black-white box. *Pharmacology Biochemistry and Behavior*, 54(1), 57-63. doi:10.1016/0091-3057(95)02130-2
- Stark, P., & Hardison, C. D. (1985). A review of multicenter controlled studies of fluoxetine vs. imipramine and placebo in outpatients with major depressive disorder. *The Journal of Clinical Psychiatry*, 46(3 Pt 2), 53.

- Stein, R. A., Jarvik, M. E., & Gorelick, D. A. (1993). Impairment of memory by fluoxetine in smokers. *Experimental and Clinical Psychopharmacology*, 1(1-4), 188-193. doi: 10.1037/1064-1297.1.1-4.188
- Stokes, P. E., & Holtz, A. (1997). Fluoxetine tenth anniversary update: The progress continues. *Clinical Therapeutics*, 19(5), 1135-1250. doi: 10.1016/S0149-2918(97)80066-5
- Tanum, L. (2000). Reboxetine: Tolerability and safety profile in patients with major depression. *Acta Psychiatrica Scandinavica*, 101(402), 37-40. doi: 10.1034/j.1600-0447.2000.02606.x
- Tanaka, M., Yoshida, M., Emoto, H., & Ishii, H. (2000). Noradrenaline systems in the hypothalamus, amygdala and locus coeruleus are involved in the provocation of anxiety: Basic studies. *European Journal of Pharmacology*, 405(1), 397-406. doi:10.1016/S0014-2999(00)00569-0
- Thase, M. E. (1997). Efficacy and tolerability of once-daily venlafaxine extended release (XR) in outpatients with major depression. The Venlafaxine XR 209 Study Group. *The Journal of Clinical Psychiatry*, 58(9), 393-398.
- Thompson, M. R., Li, K. M., Clemens, K. J., Gurtman, C. G., Hunt, G. E., Cornish, J. L., & McGregor, I. S. (2004). Chronic fluoxetine treatment partly attenuates the long-term anxiety and depressive symptoms induced by MDMA ('Ecstasy') in rats. *Neuropsychopharmacology*, 29(4), 694. doi:10.1038/sj.npp.1300347
- Timothy, C., Costall, B., & Smythe, J. W. (1999). Effects of SCH23390 and raclopride on anxiety-like behavior in rats tested in the black-white box. *Pharmacology Biochemistry and Behavior*, 62(2), 323-327. doi:10.1016/S0091-3057(98)00157-9
- Treit, D., & Fundytus, M. (1988). Thigmotaxis as a test for anxiolytic activity in rats. *Pharmacology Biochemistry and Behavior*, 31(4), 959-962. doi:10.1016/0091-3057(88)90413-3
- Troy, S. M., Lucki, I., Peirgies, A. A., Parker, V. D., Klockowski, P. M., & Chiang, S. T. (1995). Pharmacokinetic and pharmacodynamic evaluation of the potential drug interaction between venlafaxine and diazepam. *The Journal of Clinical Pharmacology*, 35(4), 410-419. doi:10.1002/j.1552-4604.1995.tb04082.x
- Troy, S. M., Turner, M. B., Unruh, M., Parker, V. D., & Chiang, S. T. (1997). Pharmacokinetic and pharmacodynamic evaluation of the potential drug interaction between venlafaxine and ethanol. *The Journal of Clinical Pharmacology*, 37(11), 1073-1081. doi:10.1002/j.1552-4604.1997.tb04290.x
- van der Staay, J. J., Kerbusch, S., & Raaijmakers, W. (1990). Genetic correlations in validating emotionality. *Behavior Genetics*, 20(1), 51-62. doi:10.1007/BF01070740

- van Erp, A. M., Kruk, M. R., Meelis, W., & Willekens-Bramer, D. C. (1994). Effect of environmental stressors on time course, variability and form of self-grooming in the rat: Handling, social contact, defeat, novelty, restraint and fur moistening. *Behavioural Brain Research*, 65(1), 47-55. doi: 10.1016/0091-3057(95)02166-3
- van Moffaert, M., Bartholome, F., Cosyns, P., De Nayer, A. R., & Mertens, C. (1995). A controlled comparison of sertraline and fluoxetine in acute and continuation treatment of major depression. *Human Psychopharmacology: Clinical and Experimental*, 10(5), 393-405. doi:10.1002/hup.470100505
- van Moffaert, M., & Dierick, M. (1999). Noradrenaline (norepinephrine) and depression. *CNS Drugs*, 12(4), 293-305. doi: 10.2165/00023210-199912040-00004
- van Praag, H. M., & Korf, J. (1971). Endogenous depressions with and without disturbances in the 5-hydroxytryptamine metabolism: A biochemical classification? *Psychopharmacologia*, 19(2), 148-152. doi: 10.1007/BF00402638
- van Praag, H., Schinder, A. F., Christie, B. R., Toni, N., Palmer, T. D., & Gage, F. H. (2002). Functional neurogenesis in the adult hippocampus. *Nature*, 415(6875), 1030-1034. doi: 10.1038/4151030a
- Veiel, H. O. (1997). A preliminary profile of neuropsychological deficits associated with major depression. *Journal of Clinical and Experimental Neuropsychology*, 19(4), 587-603. doi:10.1080/01688639708403745
- Versiani, M., Amin, M., & Chouinard, G. (2000). Double-blind, placebo-controlled study with reboxetine in inpatients with severe major depressive disorder. *Journal of Clinical Psychopharmacology*, 20(1), 28-34. doi:10.1097/00004714-200002000-00006
- Versiani, M., Mehilane, L., Gaszner, P., & Arnaud-Castiglioni, R. (1999). Reboxetine, a unique selective NRI, prevents relapse and recurrence in long-term treatment of major depressive disorder. *Journal of Clinical Psychiatry*, 60(6), 400-406. doi:10.4088/JCP.v60n0610
- Versiani, M., Moreno, R., Ramakers-van Moorsel, C. J., & Schutte, A. J. (2005). Comparison of the effects of mirtazapine and fluoxetine in severely depressed patients. *CNS Drugs*, 19(2), 137-146.
- Wadsworth, E. J., Moss, S. C., Simpson, S. A., & Smith, A. P. (2005). SSRIs and cognitive performance in a working sample. *Human Psychopharmacology: Clinical and Experimental*, 20(8), 561-572. doi: 10.1002/hup.725
- Walk, R. D. (1960). Response of dark- and light-reared rats to stimulus change. *Journal of Comparative and Physiological Psychology*, 53(6), 609-611. doi:10.1037/h0040203

- Walsh, R. N., & Cummins, R. A. (1976). The open-field test: A critical review. *Psychological Bulletin*, 83(3), 482-504. doi:10.1037/0033-2909.83.3.482
- Wang, J. W., David, D. J., Monckton, J. E., Battaglia, F., & Hen, R. (2008). Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. *The Journal of Neuroscience*, 28(6), 1374-1384. doi: 10.1523/JNEUROSCI.3632-07.2008
- Warner-Schmidt, J. L., & Duman, R. S. (2007). VEGF is an essential mediator of the neurogenic and behavioral actions of antidepressants. *Science Signaling*, 104(11), 4647. doi: 10.1073/pnas.0610282104
- Wechsler, D. (1981). *Wechsler Adult Intelligence Scale-Revised*. San Antonio, TX: The Psychological Corporation.
- Wechsler, D. (1987). *Wechsler Memory Scale-Revised*. New York: The Psychological Corporation.
- Wechsler, D. (2009). *Wechsler Memory Scale—Fourth Edition (WMS-IV) technical and interpretive manual*. San Antonio, TX: Pearson.
- Welker, W. I. (1957). "Free" versus "forced" exploration of a novel situation by rats. *Psychological Reports*, 3(g), 95-108. doi:10.2466/pr0.1957.3.g.95
- Welker, W. I. (1959). Escape, exploratory, and food-seeking responses of rats in a novel situation. *Journal of Comparative and Physiological Psychology*, 52(1), 106-111. doi:10.1037/h0042241
- Wellington, K., & Perry, C. M. (2001). Venlafaxine Extended-Release. *CNS Drugs*, 15(8), 643-669. doi: 10.2165/00023210-200115080-00007
- Wernicke, J. F., Dunlop, S. R., Dornseif, B. E., & Zerbe, R. L. (1987). Fixed-dose fluoxetine therapy for depression. *Psychopharmacology Bulletin*, 23(1), 164.
- Whimbey, A. E., & Denenberg, V. H. (1967a). Experimental programming of life histories: The factor structure underlying experimentally created individual differences. *Behaviour*, 29(2/4), 296-314. Retrieved from: <http://www.jstor.org/stable/4533195>
- Whimbey, A. E., & Denenberg, V. H. (1967b). Two independent behavioral dimensions in open-field performance. *Journal of Comparative and Physiological Psychology*, 63(3), 500-504. doi:10.1037/h0024620

- Wong, E. H., Sonders, M. S., Amara, S. G., Tinholt, P. M., Piercey, M. F., Hoffmann, W. P., ... & McArthur, R. A. (2000). Reboxetine: A pharmacologically potent, selective, and specific norepinephrine reuptake inhibitor. *Biological Psychiatry*, 47(9), 818-829. doi: 10.1016/S0006-3223(99)00291-7
- Wong, M. L., & Licinio, J. (2001). Research and treatment approaches to depression. *Nature Reviews Neuroscience*, 2(5), 343-351. doi: 10.1038/35072566
- Wyeth-Ayerst Laboratories. (2012). *Effexor XR (venlafaxine hydrochloride) extended release capsules. Prescribing information [online]*. Retrieved from <http://www.effexorxr.com>

Appendix 1

ANIMAL ETHICS COMMITTEE

Secretary, Lynda Griffioen
Email: animal-ethics@canterbury.ac.nz



AEC Ref: 2012/23R

25 June 2012

Vanessa Gray
Department of Psychology
UNIVERSITY OF CANTERBURY

Dear Vanessa

I am pleased to inform you that the Animal Ethics Committee (AEC) has approved your application entitled: "Chronic use of three types of antidepressants and their effects on memory and anxiety in male and female rats"

Approval has been granted:

- (a) for the use of 140 *Rattus norvegicus*
- (b) for your research project to be undertaken 25 June 2012 to 21 December 2012. If you require an extension of this period please contact the AEC Secretary.

As part of AEC's new Code of Ethical Conduct all applicants receiving approval to work on animals are required to provide a final report at the completion of their project. The purpose is to provide the AEC with a record of your use of animals and what was achieved by your research project. We are very much interested in your findings and to learn what you have achieved. Following the completion date indicated above you are asked to provide this report using the new Final Report form which is available at the AEC web site (<https://intranet.canterbury.ac.nz/research/ethics.shtml>).

On an annual basis the University is legally required to provide to MAF statistical data on all animal manipulations undertaken in a calendar year. To assist us in collating this information you are also required to complete and return to the AEC Secretary the attached MAF Animal Manipulation Statistical form 30 days after the completion of this project, or once every three years, whichever comes first. If no animals have been manipulated in your project please provide a "Nil" return. Please also find enclosed a copy of the Animal Welfare (Records and Statistics) Regulations 1999 for your information together with a list of Animal Type Codes and brief guideline notes for your assistance.

Yours sincerely

Associate Professor Jim Briskie
Chair
University of Canterbury Animal Ethics Committee

Appendix 2

Below are graphical representations showing the occupancy of all squares in the OFT. Corner Squares are represented by numbers 1, 4, 13 and 16. Squares next to the opening to the emergence test are represented by numbers 2 and 3.

